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(54) Title: USE OF SULFOALKYL ETHER CYCLODEXTRIN AS A PRESERVATIVE

(57) Abstract: A method of preserving formulations is provided. The method includes the step of including a derivatized cyclodextrin in a formulation capable of sustaining microbial growth. One embodiment of the formulation employs a sulfoalkyl ether cyclodextrin as a preservative and optionally as a solubilizing and complexing agent. A suitable cyclodextrin is the CAPTISOL" brand cyclodextrin (sulfobutyl ether R-cyclodextrin). Whether or not the formulation includes a conventional preservative, the formulation will remain preserved for at least a minimum predetermined period. Specific embodiments of the invention include a carrier, a derivatized cyclodextrin and optionally one or more active agents, one or more water activity-reducing agents, and/or one or more complexation-enhancing agents. The derivatized cyclodextrin reduces the water activity of the formulation. A liquid formulation can be lyophilized or otherwise dried to yield a solid formulation that is optionally reconstitutable.

Use of Sulfoalkyl Ether Cyclodextrin as a Preservative

BY:

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FIELD OF THE INVENTION

The present invention relates to a method of preserving a formulation by including a sufficient amount of derivatized cyclodextrin in the formulation, and also to preserved formulations containing a derivatized cyclodextrin at least as a preservative in the formulation.

BACKGROUND OF THE INVENTION

Preservation of formulations generally involves the use of chemical preservatives to maintain or reduce the amount or concentration of microbes in the formulation below a certain value. In the food, cosmetic and pharmaceutical industries, for example, conventional preservatives are included to inhibit the proliferation of or reduce the rate of proliferation of microbes in the respective products.

Many compounds varying dramatically in structure are known to serve as conventional preservatives. Depending upon the intended application/use of a product, a particular preservative is generally preferred. For example, conventional preservatives for food use are generally different than preservatives for cosmetic use which in turn are generally different than preservatives for pharmaceutical use. Exemplary conventional preservatives include benzalkonium chloride, benzethonium chloride, benzoic acid, chlorobutanol, chlorocresol, methyl, ethyl and propyl parabens, phenol, phenoxyethanol, propyl gallate, sorbic acid, benzyl alcohol, EDTA, pentetate, azide, organic solvent (such as glycol, propylene glycol, or polyethylene glycol), peroxide, ozone, chlorite, sodium bisulfite, potassium metabisulfite, potassium sulfite, sodium sulfite, and others known to those of ordinary skill in the art.

Some pharmaceutical formulations, especially those formulations comprising a medium that sustains microbial growth, are particularly prone to contamination by microbes. Exemplary formulations include those containing water, carbohydrate, lipid, protein, a biodegradable substance or other similar formulations. In particular, albumin-

based and lipid-based formulations are particularly prone to contamination by microbes and always require a conventional preservative in order to maintain an acceptable shelf life.

Lipid-based emulsion formulations are typically problematic with regard to microbial growth, not only because the lipid components can readily support the growth, but because 0.22 μm or smaller "sterilizing" filters cannot be used. A filter pore size of ≥5 μm is generally recommended for emulsion formulations.

Cyclodextrins are cyclic carbohydrates derived from starch. The unmodified cyclodextrins differ by the number of glucopyranose units joined together in the cylindrical structure. The parent cyclodextrins contain 6, 7, or 8 glucopyranose units and are referred to as α-, β-, and γ-cyclodextrin respectively. Each cyclodextrin subunit has secondary hydroxyl groups at the 2 and 3 positions and a primary hydroxyl group at the 6-position. The cyclodextrins may be pictured as hollow truncated cones with hydrophilic exterior surfaces and hydrophobic interior cavities. In aqueous solutions, these hydrophobic cavities provide a haven for hydrophobic organic compounds that can fit all or part of their structure into these cavities. This process, known as inclusion complexation, may result in increased apparent aqueous solubility and stability for the complexed drug. The complex is stabilized by hydrophobic interactions and does not involve the formation of any covalent bonds.

This dynamic and reversible equilibrium process can be described by Equations 1 and 2, where the amount in the complexed form is a function of the concentrations of the drug and cyclodextrin, and the equilibrium or binding constant, K_b. When cyclodextrin formulations are administered by injection into the blood stream, the complex rapidly dissociates due to the effects of dilution and non-specific binding of the drug to blood and tissue components.

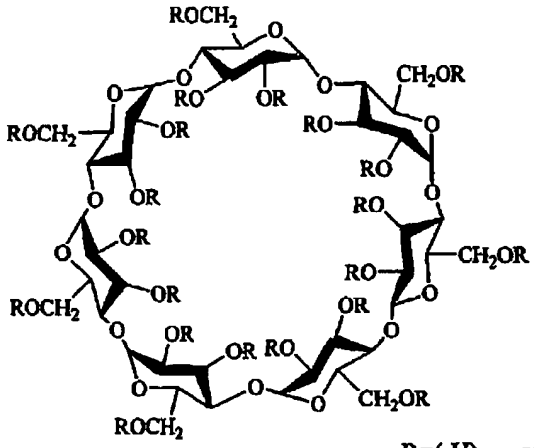
$$\text{Drug} + \text{Cyclodextrin} \xleftrightarrow{K_b} \text{Complex} \qquad \text{Equation 1}$$

$$K_b = \frac{[\text{Complex}]}{[\text{Drug}][\text{Cyclodextrin}]} \qquad \text{Equation 2}$$

Binding constants of cyclodextrin and an active agent can be determined by the equilibrium solubility technique (T. Higuchi et al. in "Advances in Analytical Chemistry

and Instrumentation Vol. 4"; C.N. Reilly ed.; John Wiley & Sons, Inc, 1965, pp. 117-212). Generally, the higher the concentration of cyclodextrin, the more the equilibrium process of Equations 1 and 2 is shifted to the formation of more complex, meaning that the concentration of free drug is generally decreased by increasing the concentration of cyclodextrin in solution.

The underivatized parent cyclodextrins are known to interact with human tissues and extract cholesterol and other membrane components, particularly upon accumulation in the kidney tubule cells, leading to toxic and sometimes fatal renal effects. Chemical modification of the parent cyclodextrins (usually at the hydroxyls) has resulted in derivatives with improved safety while retaining or improving the complexation ability. Of the numerous derivatized cyclodextrins prepared to date, only two appear to be commercially viable: the 2-hydroxypropyl derivatives (HP-CD; neutral cyclodextrins being commercially developed by Janssen and others), and the sulfoalkyl ether derivatives, such as sulfobutyl ether, (SBE-CD; anionic cyclodextrins being developed by CyDex, Inc.) However, the HP-β-CD still possesses toxicity that the SBE-CD does not.



R=(-H)$_{21-n}$ or (-(CH$_2$)$_4$-SO$_3$Na)$_n$
where n=6.0-7.1

Sulfobutyl Ether-β-Cyclodextrin (Captisol®)

A sulfobutyl ether derivative of beta cyclodextrin (SBE-β-CD), in particular the derivative with an average of about 7 substituents per cyclodextrin molecule, is being commercialized by CyDex, Inc. as CAPTISOL®. The anionic sulfobutyl ether substituent dramatically improves the aqueous solubility of the parent cyclodextrin. In addition, the

presence of the charges decreases the ability of the molecule to complex with cholesterol as compared to the hydroxypropyl derivative. Reversible, non-covalent, complexation of drugs with CAPTISOL® cyclodextrin generally allows for increased solubility and stability of drugs in aqueous solutions. While CAPTISOL ® cyclodextrin is a relatively new but known cyclodextrin, its use as a preservative has not previously been evaluated.

Hemolytic assays are generally used in the field of parenteral formulations to predict whether or not a particular formulation is likely to be unsuitable for injection into the bloodstream of a subject. If the formulation being tested induces a significant amount of hemolysis, that formulation will generally be considered unsuitable for administration to a subject. It is generally expected that a higher osmolality is associated with a higher hemolytic potential. As depicted in FIG. 1 (Thompson, D.O., *Critical Reviews in Therapeutic Drug Carrier Systems*, (1997), 14(1), 1-104), the hemolytic behavior of the CAPTISOL® cyclodextrin is compared to the same for the parent β-cyclodextrin, the commercially available hydroxypropyl derivatives, ENCAPSIN™ cyclodextrin (degree of substitution~3-4) and MOLECUSOL® cyclodextrin (degree of substitution~7-8), and two other sulfobutyl ether derivatives, SBE1-β-CD and SBE4-β-CD. Unlike the other cyclodextrin derivatives, sulfoalkyl ether (SAE-CD) derivatives, in particular those such as the CAPTISOL® cyclodextrin (degree of substitution~7) and SBE4-β-CD (degree of substitution~4), show essentially no hemolytic behavior and exhibit substantially lower membrane damaging potential than the commercially available hydroxypropyl derivatives at concentrations typically used to solubilize pharmaceutical formulations. The range of concentrations depicted in the figure includes the concentrations typically used to solubilize pharmaceutical formulations and provide preserving activity when initially diluted in the blood stream after injection.

The osmolality of a formulation is generally associated with its hemolytic potential: the higher the osmolality (or the more hypertonic), the greater the hemolytic potential. Zannou et al. ("Osmotic properties of sulfobutyl ether and hydroxypropyl cyclodextrins", *Pharma. Res.* (2001), 18(8), 1226-1231) compared the osmolality of solutions containing SBE-CD and HP-CD. As depicted in FIG. 2, the SBE-CD containing solutions have a greater osmolality than HP-CD containing solutions comprising similar concentrations of cyclodextrin derivative. Thus, it is surprising that SAE-CD exhibits lower hemolysis than does HP-CD at equivalent concentrations, even though HP-CD has a lower osmolality.

Methylated cyclodextrins have been prepared and their hemolytic effect on human erythrocytes has been evaluated. These cyclodextrins were found to cause moderate to severe hemolysis (Jodal et al., *Proc. 4th Int. Symp. Cyclodextrins*, (1988), 421-425; Yoshida et al., *Int. J. Pharm.*, (1988), 46(3), 217-222).

Cyclodextrins have been used widely in all different types of cosmetic, food and pharmaceutical formulations. The underivatized α-cyclodextrin and β-cyclodextrin are the most widely used cyclodextrins. Cyclodextrins, however, are not generally known for their ability to preserve formulations. For this reason, preservatives are typically added to cyclodextrin containing formulations. Most preservatives complex to some degree with cyclodextrins, and in some cases, only the uncomplexed fraction is available to act as a preservative. However, since many of the preservatives are poorly soluble in aqueous systems, the complexation can assist in providing constant levels of the preservative in the formulation.

Japanese Patent Application JP 2001029054 discloses a food formulation comprising a complex of cyclodextrin and a volatile preservative. Japanese Patent Application JP 2002029901 discloses a film used for food packaging, wherein the film comprises a known preservative complexed with a cyclodextrin. Japanese Patent Application JP 2002281894 discloses a preservative comprising an inclusion complex of hinokitiol and cyclodextrin for use in lining packages of fruits and vegetables. Japanese Patent Applications JP 2000004854, JP 11089548, JP 11130608, JP 10147380, and JP 09299458 disclose a preservative comprising an inclusion complex of isothiocyanate and cyclodextrin for use in lining packages of food. Japanese Patent Application JP 2000302601 discloses a preservative comprising an inclusion complex of 2-octyl-4-isothiazolin-3-one and cyclodextrin for use in a sprayable paint. European Patent Application EP 895718 to Wimmer et al. discloses a microbicidal formulation comprising an isothiazolinone and β-cyclodextrin complex. Japanese Patent Application JP 11279139 discloses a complex comprising diiodomethyl p-tolyl sulfone with methyl-β-cyclodextrin as a microbicidal agent. Japanese Patent Application JP 11276135 discloses a film for preserving, wherein the film includes a complex comprising thiamine lauryl sulfate and maltosyl cyclodextrin. Japanese Patent Application JP 11228302 discloses a complex comprising 2-(4-thiazolyl)benzimidazole (a preservative) and a cyclodextrin for preserving food. Japanese Patent Application JP 11151288 discloses a complex comprising a silver-protein complex complexed with hydroxypropyl cyclodextrin for use

in a solution for contact lenses. The use of a complex of cinnamic acid and cyclodextrin as a preservative is disclosed in Japanese Patent Applications No. JP 10273402 and JP 10273401. A preservative complex comprising halocyanoacetamide and β-cyclodextrin is disclosed in Japanese Patent Application No. JP 07277908. European Patent Application No. EP 621287 discloses a preserved diagnostic test kit comprising a complex of a derivatized cyclodextrin, such HPCD or DMCD, wherein the complexes are more active against gram-positive and gram-negative bacteria than the biocide alone.

PCT International Publication No. WO 9806381 discloses a preservative formulation for use in aqueous pharmaceutical compositions. The system comprises boric acid and one or more compounds selected from the group consisting of C16 benzalkonium halide compounds, polymeric quaternary ammonium compounds, and quaternary ammonium alkylene glycol phospholipid derivatives such as [R1C(O)XR2N+(R3)(R4)Y-CH(OH)CH_2O]aP(O)(OH)b (a + b = 3; R1 = C8-22 alkyl or alkene; X = NH, O, CH_2; R2 = C2-6 alkyl; each R3 is independently C1-12 alkyl or alkene; and Y is nothing or C1-6 alkyl or alkene) and pharmaceutically acceptable salts thereof. The formulation also includes hydroxypropyl β-cyclodextrin.

PCT International Publication No. WO 9710805 discloses a preservative composition for ophthalmic use. The composition comprises a cyclodextrin, a quaternary ammonium salt, an alkylene glycol and a drug. Japanese Patent Application No. JP 01016728 disclose an ophthalmic solution containing a preservative comprising a cationic surfactant, such as benzalkonium chloride, and a cyclodextrin.

PCT International Publication No. WO 9530425 discloses a preservative formulation for use in aqueous pharmaceutical compositions. The composition comprises a poly(benzalkonium) salt, drug, hydroxypropyl-β-cyclodextrin, EDTA and salt.

Preservative formulations containing volatile preservatives that have been complexed with a cyclodextrin are disclosed in Japanese Patent Applications No. JP 08143404 and JP 08245302.

Japanese Patent Application No. 05163104 discloses inclusion compounds of cyclodextrin with 2,2-dibromo-2-nitroethanol (I), 1,1-dibromo-1-nitro-2-acetoxyethane, 2-bromo-2-nitro-1,3-diacetoxypropane, 2-(thiocyanomethylthio)-benzothiazole, 2-bromo-2-nitropropane-1,3-diol, or a mixture of ~80% 4-(2-nitrobutyl)-morpholine and ~20% 4,4'-(2-ethyl-2-nitrotrimethylene)dimorpholine as industrial microbicides. The mixture reportedly inhibits the growth of *Bacillus subtilis, Escherichia coli, Klebsiella*

pneumoniae, *Aspergillus niger*, and *Penicillium citrinum* with minimum inhibitory concentrations of 66, 66, 66, 66, and 33 ppm, resp.

Japanese Patent Application No. JP 02268643 discloses a preservative composition for food. The composition comprises an inclusion complex of quercetin and β-cyclodextrin.

Cyclodextrins can also be mixed with other known preservatives that may or may not complex with the cyclodextrin to form a preservative. Chinese Patent Application No. CN 1336145 discloses a complex concoction comprising cyclodextrin, an antibiotic agent and a conventional preservative. PCT International Publication No. WO 2000012137 discloses an ophthalmic formulation comprising chlorine dioxide and sulfobutyl ether cyclodextrin complex among other things. Japanese Patent Application No. JP 05308897 discloses a method of preserving protein-based foods by applying a cyclodextrin, optionally with NaCl or $MgCl_2$, onto the surface of the food. Japanese Patent Application No. JP 06181728 discloses a method of preserving foods, such as tea and rice, by mixing under pressure a cyclodextrin with the food and packaging the food in a moisture-proof sealed container. Japanese Patent Application No. JP 07313129 discloses a method of preserving foods by applying a ferulic acid-cyclodextrin complex and maintaining the pH between about 3-10.

The affect that a cyclodextrin has on the preserving capacity of a known preservative is relatively unpredictable. Lehner et al. (*J. Pharmacy Pharmacology* (1994), 46(3), 186-191) report on the effect that hydroxypropyl cyclodextrin has upon the activity of known preservatives. Apparently, the greater the amount of preservative bound to the cyclodextrin, the lower the preserving activity of the preservative. This concept is the premis of the technology disclosed in U.S. Pregrant Publication No. 20020076449, which discloses cyclodextrin containing formulations containing preservative agents that have a reduced binding to the cyclodextrin. On the other hand, European Patent Application No. EP 621287 discloses a preservative solution useful for the preservation of diagnostic test kits. The solution comprises a complex of a derivatized cyclodextrin, e.g., hydroxypropyl α- or γ-cyclodextrin or Me-β-cyclodextrin, with a biocide (o-phenylphenol, Densil P, Proxel, methylenebisthiocyanate, etc.) having solubility ≤0.15 wt./vol.% at 25°C. They report that the complexes are significantly more active against gram-pos. and gram-neg. bacteria than the biocides alone. Simpson (*FEMS Microbiology Letters* (1992), 90(2), 197-199) reports that cyclodextrins can be complexed with quaternary ammonium

biocides to inactive their antimicrobial activity. Loftsson et al. (*Drug Development Indust. Pharm.* (1992), 18(13), 1477-84) and Lehner et al. (*International J. Pharmaceutics* (1993), 93(1-3), 201-208) disclose that hydroxypropyl cyclodextrin reduces the antimicrobial activity of a range of preservatives, and the preservatives displace a drug that is complexed with the cyclodextrin thereby reducing the solubilizing effect of the cyclodextrin.

Since cyclodextrins are carbohydrate-based, they too might serve as a food source for microbes. Indeed, Japanese Patent Application JP 2001112465 discloses a method of promoting the growth of microorganisms by including a natural or derivatized cyclodextrin and another polysaccharide in the medium.

Sikorski et al. (U.S. Pregrant Patent Application Publication No. 2002/0187960) discloses a method of inhibiting microbial growth in liquid formulations containing cyclodextrin. Sikorski et al. require that the formulations, which may contain HPCD, possess an alkaline pH or an added antimicrobial agent.

Water availability is one of the most important factors affecting the growth of microorganisms and is generally expressed in physical terms as water activity. Water activity, abbreviated a_w, is a ratio of the water vapor pressure of the air over the substance or solution divided by the vapor pressure of pure water at the same temperature ($a_w = P_{product} / P_{water}$). Thus the value of a_w varies between 0 and 1. Bacteria, yeasts, and molds require a certain amount of available water to support growth. Therefore, by lowering the water activity the proliferation of these microorganisms can be controlled. Growth of most bacteria can be stopped by lowering the water activity to 0.80. Yeasts and molds tend to be more resistant to water activity and therefore it is generally necessary to lower the water activity to 0.70-0.75 to prevent growth. More specifically, the water activity range generally recognized as the lower limit necessary to prevent the growth of the microorganisms commonly used for challenging pharmaceutical products during preservative effectiveness testing are listed in the table below.

Microorganism	a_w range
Pseudomonas aeruginosa	0.97-0.95
Escherichia coli	0.96-0.95
Staphylococcus aureus	0.86-0.80
Candida albicans	0.87
Aspergillus niger	0.80-0.77

The minimum a_w for growth of the microorganisms is generally determined by the addition of salt. It is recognized that the minimum a_w values for growth prevention with other solutes may be different.

Lowering the a_w of a product can be thought of as "binding-up" moisture. In other words, water is still present, however, it is just not readily available to be used for biological processes. Thus, substantial moisture can stay in the product, as long as it is bound-up somehow. Binding of the free water can be achieved by the addition of solutes such as glycerin, alcohol, sugar, sugar-alcohol, salt or sugar-salt combinations.

When a bacterial cell is placed in a solution with low water activity, the cell dehydrates and bacterial growth is inhibited. It is generally recognized, according to the principles of thermodynamics, that water activity is the driving force behind dehydration. Donald Orth, Ph.D., in the book Preservative-Free and Self-Preserving Cosmetics and Drugs (1997, Marcel Dekker, New York), discusses various different conventional preservatives and a "preservative system." Lehner et al. (*Pharm. Ind.* (1994), 56(10), 911-914) disclose the biostatic/biocidal activity of HPCD.

Humectants and other water activity-reducing agents are often added to food, drug or cosmetic formulations in order to reduce the water activity of and preserve the formulations. A number of scientific publications discuss the effect that poly(ethylene glycol) has upon the water activity of aqueous compositions. Ambrose et al. (*Antimicrobial Agents and Chemotherapy* (1991), 35(9), 1799-1803) disclose a composition comprising sucrose or xylose and PEG 400 and H_2O_2. An antibacterial effect against *Staphylococcus aureus* was observed in the presence and absence of H_2O_2. Eliassi et al. (*Journal of Chemical and Engineering Data* (1999), 44(1), 52-55; *European Polymer Journal* (2001), 37(7), 1487-1492) and Ninni et al. (*Thermochimica Acta*, (22 MAR 1999), 328(1-2), 169-176) disclose the results of studies on the water activity of solutions containing differing amounts of PEG. Vaamonde et al. (*J. Food Sci.*, (1982) 47(4), 1259-1262; *J. Food Sci.* (1984), 49(1), 296-297) disclose the results of studies on the effect that solutes such as l-proline, l-lysine, β-alanine, sorbitol, KCl, sodium lactate and PEG have on the growth of *S. aureus*. No growth was observed in any case when the water activity was at or slightly below a_w 0.86. Chirife et al. (*Antimicrobial Agents and Chemotherapy*, (1983 Sep) 24(3), 409-412) disclose that concentrated polyethylene glycol 400 solutions have significant antibacterial activity against various pathogenic bacteria including *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and

Staphylococcus aureus. Voilley et al. (*Lebensmittel-Wissenschaft und -Technologie* (1989), 22(1), 32-38) disclose the results on the reduction of water activity in aqueous solutions of sugars (glucose, maltose, maltotriose) or PEG 35,000.

Although artisans have recognized that poly(vinylpyrrolidone) (PVP) and poly (ethylene glycols) can have an impact on the water activity of aqueous solutions, no work has been done to evaluate their water activity in combination with cyclodextrins, or their potential use as a preservative. Jellinek et al. (*Kolloid Z. Z. Polym.* (1967), 220(2), 122-133) disclose the results of studies on the effect that PVP has upon freezing of water.

Given the fact that many conventional preservatives complex with cyclodextrins, it would be extremely beneficial to identify a cyclodextrin derivative that possesses biostatic, (inhibiting the growth of microorganisms), or biocidal, (destroying microorganisms,) properties on its own, i.e., in the absence of a conventional preservative. As noted above, in the field of preservatives for cosmetic, food or pharmaceutical use, there has not been identified any particular cyclodextrin derivative that possesses antimicrobial activity in aqueous solutions in the absence of an additional added conventional preservative. A need remains in the art for simplified aqueous formulations that remain preserved in the absence of a conventional preservative. A need also remains for a preserved pharmaceutical formulation containing a cyclodextrin complexed with a drug, wherein the formulation excludes a conventional pharmaceutical preservative. A need also remains for a formulation comprising an active agent, a cyclodextrin and a conventional preservative, wherein the cyclodextrin does not reduce the activity of the preservative or wherein the antimicrobial activity of the formulation is not reduced.

SUMMARY OF THE INVENTION

The present invention seeks to overcome the disadvantages present in known formulations. As such, a derivatized cyclodextrin-based, e.g., sulfoalkyl ether cyclodextrin (SAE-CD)-based, preserved formulation is provided. The present formulation can include any known active agent. The formulation is preserved in terms of its ability to resist microbial contamination, i.e., it has a reduced potential for sustaining bacterial growth as compared to a similar formulation that excludes the SAE-CD and a conventional preservative. The present formulation may provide enhanced solubility and/or enhanced chemical, thermochemical, hydrolytic and/or photochemical stability of

the active agent or other ingredients in the formulation. Moreover, the present formulation possesses other physicochemical advantages over other formulations.

The formulation of the invention includes underivatized cyclodextrin and/or derivatized cyclodextrin in an amount sufficient to reduce the water activity of the formulation to a value below which a target microbe will not be able to grow and/or will be killed. In the absence of a conventional preservative or other agent(s) that enhance(s) or improve(s) the biostatic or biocidal activity of the cyclodextrin, a formulation of the invention will have a water activity (a_w) less than about 0.97±0.025. Another biostatic, biocidal, or water activity reducing-agent can be included in the formulation to provide further improved preserving capacity.

An SAE-CD-containing formulation can be prepared with sufficient active agent solubility and stability for a commercial product. If needed, the SAE-CD-containing formulation can be prepared as a clear aqueous solution that can be sterile filtered through a filter having a pore size of 0.45 μm or less and that is stable and preserved under a variety of storage conditions. An SAE-CD-containing liquid formulation can also be converted to a solid or powder for reconstitution.

One aspect of the invention provides a liquid formulation comprising an effective amount of an active agent and an SAE-CD, wherein the SAE-CD is present in an amount sufficient to preserve the formulation during storage. The SAE-CD can be present in less than stoichiometric, stoichiometric or greater than stoichiometric amounts with respect to the amount of active agent present in the formulation. The SAE-CD need not complex with the active agent. The SAE-CD need not form ionic associations with the active agent. The formulation can comprise more than one active agent.

Specific embodiments of the invention include those wherein: 1) the active agent to SAE-CD molar ratio is less than one, about one or greater than one; 2) the SAE-CD is sulfobutyl ether 4-β-CD or sulfobutyl ether 7-β-CD; 3) the SAE-CD is a compound of the formula 1 or a mixture thereof; 4) the formulation further comprises a conventional preservative , an antioxidant, a buffering agent, an acidifying agent, a solubilizing agent, a complexation enhancing agent, saline, an electrolyte, another therapeutic agent, an alkalizing agent, a humectant or a combination thereof; 5) the SAE-CD is present in an amount sufficient to provide a clear solution; 6) the liquid formulation is lyophilized or otherwise dried to form a solid formulation for reconstitution; 7) the formulation comprises at least 4.8±0.5% wt./vol of SAE-CD to provide biostasis; 8) the formulation

comprises more than one active agent, wherein each active agent may or may not complex with the SAE-CD; 8) the formulation comprises an excess, on a molar basis, of SAE-CD; 9) the formulation comprises an excess, on a molar basis, of active agent; 10) the formulation has been purged with an inert gas to remove substantially all of the oxygen contained in the formulation; 11) the formulation further comprises one or more conventional preservatives; 12) at least one active agent present in the formulation has a greater binding with the SAE-CD than does a conventional preservative in the formulation; 13) the water activity of the formulation is less than about 0.97±0.025 as measured according to the procedures detailed herein; 14) the formulation further comprises a liquid carrier other than water; 15) the formulation is a liquid; 16) the formulation is a semi-solid; 17) the formulation is a solid; 18) the formulation has been prepared at a temperature at or above 5°C, at or above 25°C, at or above 35°C, at or above 45°C or at or above 50°C; 19) the formulation has been prepared at a temperature approximating ambient temperature; 20) the SAE-CD, or derivatized cyclodextrin, reduces the water activity of the formulation; 21) the formulation comprises at least 25.6±2.5% wt./vol of SAE-CD to provide biocidal properties; 22) the SAE-CD is present in an amount sufficient to unmask the biocidal or biostatic properties of an active agent, which properties are masked when the active agent is present in another formulation.

The water activity of the formulation is generally reduced to less than about 0.98±0.019, less than 0.97±0.025, less than 0.96±0.025, less than 0.95±0.01, less than 0.925, less than 0.90 or less than 0.80. The preferred water activity value may vary according to the components present in the formulation. The observed water activity value can also vary according to the instrument used to measure it as well as the calibration of the instrument and reproducibility of measurements (as expressed by standard deviation) taken by the instrument.

The invention also provides a liquid formulation that can be sterile filtered, wherein the formulation comprises a liquid carrier, an SAE-CD and one or more active agents.

The invention also provides methods of preparing a liquid formulation. A first method comprises the steps of: forming a first aqueous solution comprising a cyclodextrin derivative; forming a second solution comprising active agent; and mixing the first and second solutions to form the liquid formulation. A second method is similar to the first step except that the active agent is added directly to the first solution without formation of

the second solution. A third method is similar to the first except that the cyclodextrin derivative is added directly to the second solution without formation of the first solution. A fourth method comprises the steps of: adding a solution comprising active agent to a powdered or particulate cyclodextrin derivative. A fifth method comprises the steps of: adding the active agent directly to the powdered or particulate cyclodextrin derivative; and adding a second solution. A sixth method comprises the steps of: creating the liquid formulation by any of the above methods and then isolating a solid material by lyophilization, spray-drying, spray-freeze-drying, antisolvent precipitation, a process utilizing a supercritical or near supercritical fluid, or other methods known to those of ordinary skill in the art to make a powder for reconstitution.

Specific embodiments of the methods of preparing a liquid formulation include those wherein: 1) the method further comprises the step of sterile filtering the formulation through a filtration medium having a pore size of 0.1 microns or larger; 2) the liquid formulation is sterilized by irradiation or autoclaving; 3) the method further comprises the step of isolating a solid from the solution; 4) the solution is purged with nitrogen or argon or other inert pharmaceutically acceptable gas such that a substantial portion of the oxygen dissolved in, and/or in surface contact with the solution is removed.

Still another aspect of the invention provides a reconstitutable solid pharmaceutical composition comprising an active agent, a cyclodextrin derivative and optionally at least one other pharmaceutical excipient. When this composition is reconstituted with an aqueous liquid to form a preserved liquid formulation, it can be administered by injection, infusion, topically, by inhalation or orally to a subject.

The invention also provides a method of unmasking a preservative property of a drug not otherwise known to possess such property, the method comprising the step of exposing the drug to SAE-CD in an aqueous solution contaminated with a microbe, wherein the SAE-CD is present in an amount sufficient to unmask a preservative property of the drug, and the drug is present in an amount sufficient to provide a preservative effect in the solution.

Specific embodiments of the reconstitutable solid pharmaceutical composition includes those wherein: 1) the composition comprises an admixture of a solid SAE-CD and active agent-containing solid comprising an active agent and optionally at least one solid pharmaceutical excipient, such that a major portion of the active agent is not complexed with the SAE-CD prior to reconstitution; and/or 2) the composition comprises

a solid mixture of an SAE-CD and an active agent, wherein a major portion of the active agent is complexed with the SAE-CD prior to reconstitution.

The invention also provides a method of preserving a formulation, the method comprising the step of including an SAE-CD in the formulation in an amount sufficient to preserve the formulation. When compared to a control formulation excluding the SAE-CD, a preserved formulation containing SAE-CD will have a lower bioburden than the control formulation after similar storage time and conditions. In specific embodiments, the bioburden of the SAE-CD containing formulation after incubation is the same as or less than its initial bioburden after contamination.

Specific embodiments of the method include those wherein: 1) the formulation is an aqueous formulation and the SAE-CD is present in an amount of at least about 25 %wt./vol of the formulation for biocidal activity and at least 4.8% w/v for biostasis; 2) the SAE-CD is present as an alkali metal salt; 3) the method further comprises the step of including one or more active agents in the formulation; 4) the method further comprises the step of including one or more conventional preservatives in the formulation; 5) the method further comprises the step of sterilizing the formulation after addition of the SAE-CD and prior to storage of the formulation; 6) the method further comprises the step of including one or more humectants in the formulation; 7) the method further comprises the step of reducing the water activity of the formulation by addition of the SAE-CD; 8) the method further comprises the step of including one or more water activity-reducing agents in the formulation; 9) the formulation has a pH in the range of about 1-11; 10) the water activity of the composition is less than about 0.99 as measured according to the procedures detailed herein; 11) the method further comprises the step of preparing the formulation at a temperature at or above 5°C, at or above 25°C, at or above 35°C, at or above 45°C or at or above 50°C; 12) the derivatized cyclodextrin is SAE-CD, HPCD, a water soluble derivatized cyclodextrin capable of reducing the water activity of the composition or a mixture thereof.

The invention also provides a method of reducing the water activity of an aqueous composition, the method comprising the step of including a derivatized cyclodextrin in the aqueous composition at a concentration sufficient to reduce the water activity.

These and other aspects of this invention will be apparent upon reference to the following detailed description, examples, claims and attached figures.

BRIEF DESCRIPTION OF THE FIGURES

The following drawings are given by way of illustration only, and thus are not intended to limit the scope of the present invention.

Figure 1 depicts a graph of the hemolytic behavior of the CAPTISOL® cyclodextrin as compared to the same for the parent β-cyclodextrin, the commercially available hydroxypropyl derivatives, ENCAPSIN™ cyclodextrin (degree of substitution ~3-4) and MOLECUSOL® cyclodextrin (degree of substitution ~7-8), and two other sulfobutyl ether derivatives, SBE1-β-CD and SBE4-β-CD.

Figure 2 depicts a graph of the osmolality of SBE-CD containing solutions of various degrees of substitution and HP-CD containing solutions comprising similar concentrations of cyclodextrin derivative.

Figure 3 depicts a graph of the relationship between osmolality and concentration of SBE-CD in an unbuffered aqueous solution at room temperature.

Figure 4 depicts a graph of the relationship between concentration of various cyclodextrin derivatives and resulting water activity in aqueous media.

Figure 5 depicts a graph of the relationship between concentration of SAE-CD with differing degrees of substitution and the resulting water activity in aqueous media.

Figure 6 depicts a graph of the relationship between concentration of material and the resulting water activity in aqueous media. A combination of SAE-CD and PEG is included in each of the solutions in the graph.

Figure 7 depicts a graph of the relationship between concentration of material and the resulting water activity in aqueous media. A combination of SAE-CD and PVP-12 is included in each of the solutions in the graph.

Figure 8 depicts a graph of the relationship between concentration of material and the resulting water activity in aqueous media. A combination of SAE-CD and PVP-17 is included in each of the solutions in the graph.

DETAILED DESCRIPTION OF THE INVENTION

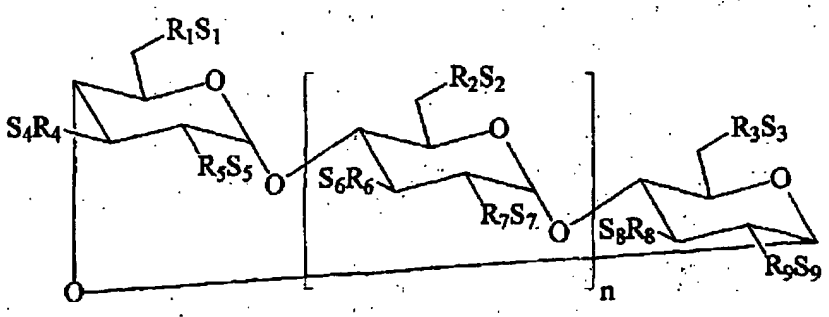
A preserved formulation of the invention comprising a derivatized cyclodextrin, a carrier and one or more active agents provides unexpected advantages over other similar formulations that exclude the derivatized cyclodextrin. The presently claimed formulation overcomes many of the undesired properties of other known preserved formulations. The

derivatized cyclodextrin serves to preserve the formulation from microbial contamination and/or proliferation.

The preserved formulation can be a liquid, solid, suspension, powder, gel, cream, ointment, paste, stick, tablet, capsule, osmotic device, dispersion, emulsion, patch or any other type of formulation requiring preservation.

The formulation of the invention can employ different means of stopping or inhibiting an increase in bioburden. In other words, it can employ a derivatized cyclodextrin and one or more preservatives in order to form a preserved formulation. One reason for doing so is that though a microorganism might survive a single preservative challenge relatively well, the energy expenditure required to meet the first challenge leaves it exposed and vulnerable to attack from another preservative. For example, under ideal conditions, 10% sodium chloride is required to inhibit Clostridium botulinum via reduction in water activity. However, at pH 5.4-5.8 in the presence of only 100ppm nitrate, only 3.5% sodium chloride is required to preserve the system. Another reason is that some microorganisms may develop resistance to a particular approach of preservation. Thus a combination of preservative approaches might be required to preserve a preparation from contamination by several microbes.

The present invention provides SAE-CD based formulations, wherein the SAE-CD is a compound of the *Formula 1:*



Formula 1

wherein:

n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of R_1 and R_2 is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, preferably a -O-$(CH_2)_mSO_3^-$ group, wherein m is 2 to 6, preferably 2 to 4, (e.g. -$OCH_2CH_2CH_2SO_3^-$ or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation which includes, for example, H^+, alkali metals (e.g. Li^+, Na^+, K^+), alkaline earth metals (e.g., Ca^{+2}, Mg^{+2}), ammonium ions and amine cations such as the cations of (C_1 - C_6)- alkylamines, piperidine, pyrazine, (C_1 - C_6)-alkanolamine and (C_4 - C_8)-cycloalkanolamine.

The SAE-CD used is described in U.S. Patents No. 5,376,645 and No. 5,134,127 to Stella et al, the entire disclosures of which are hereby incorporated by reference. U.S. Patent No. 3,426,011 to Parmerter et al. discloses anionic cyclodextrin derivatives having sulfoalkyl ether substituents. Lammers et al. (*Recl. Trav. Chim. Pays-Bas* (1972), 91(6), 733-742); *Staerke* (1971), 23(5), 167-171) and Qu et al. (*J. Inclusion Phenom. Macro. Chem.*, (2002), 43, 213-221) disclose sulfoalkyl ether derivatized cyclodextrins. An SAE-CD can be made according to the disclosures of Stella et al., Parmerter et al., Lammers et al. or Qu et al., and if purified to remove the major portion of the underivatized parent cyclodextrin, used as a preservative according to the present invention.

The terms "alkylene" and "alkyl," as used herein (e.g., in the -O-(C_2 - C_6-alkylene)SO_3^- group or in the alkylamines), include linear, cyclic, and branched, saturated and unsaturated (i.e., containing one double bond) divalent alkylene groups and monovalent alkyl groups, respectively. The term "alkanol" in this text likewise includes both linear, cyclic and branched, saturated and unsaturated alkyl components of the alkanol groups, in which the hydroxyl groups may be situated at any position on the alkyl moiety. The term "cycloalkanol" includes unsubstituted or substituted (e.g., by methyl or ethyl) cyclic alcohols.

An embodiment of the present invention provides compositions containing a mixture of cyclodextrin derivatives, having the structure set out in formula (I), where the composition overall contains on the average at least 1 and up to 3n + 6 alkylsulfonic acid moieties per cyclodextrin molecule. The present invention also provides compositions containing a single type of cyclodextrin derivative, or at least 50% of a single type of cyclodextrin derivative. The invention also includes formulations containing cyclodextrin derivatives having a narrow or wide and high or low degree of substitution. These combinations can be optimized as needed to provide cyclodextrins having particular properties.

The present invention also provides compositions containing a mixture of cyclodextrin derivatives wherein two or more different types of cyclodextrin derivatives

are included in the composition. By different types, is meant cyclodextrins derivatized with different types of functional groups e.g., hydroxyalkyl and sulfoalkyl, and not to the heterogeneous nature of derivatized cyclodextrins due to their varying degrees of substitution. Each independent different type can contain one or more functional groups, e.g. SBE-CD where the cyclodextrin ring has only sulfobutyl functional groups, and hydroxypropyl-ethyl-B-CD where the cyclodextrin ring has both hydroxypropyl functional groups and ethyl functional groups. The amount of each type of cyclodextrin derivative present can be varied as desired to provide a mixture having the desired properties.

Exemplary SAE-CD derivatives include SBE4-β-CD, SBE7-β-CD, SBE11-β-CD, and SBE4-γ-CD which correspond to SAE-CD derivatives of the formula I wherein n = 5, 5, 5 and 6; m is 4; and there are 4, 7, 11 and 4 sulfoalkyl ether substituents present, respectively. It has been found that these SAE-CD derivatives increase the solubility of poorly water soluble active agents to varying degrees.

Since SAE-CD is a poly-anionic cyclodextrin, it can be provided in different salt forms. Suitable counterions include cationic organic atoms or molecules and cationic inorganic atoms or molecules. The SAE-CD can include a single type of counterion or a mixture of different counterions. The properties of the SAE-CD can be modified by changing the identity of the counterion present. For example, a first salt form of SAE-CD can have a greater water activity reducing power than a different second salt form of SAE-CD. Likewise, an SAE-CD having a first degree of substitution can have a greater water activity reducing power than a second SAE-CD having a different degree of substitution.

By "complexed" is meant "being part of a clathrate or inclusion complex with", i.e., a complexed therapeutic agent is part of a clathrate or inclusion complex with a cyclodextrin derivative. By "major portion" is meant at least about 50% by weight. Thus, a formulation according to the present invention may contain an active agent of which more than about 50% by weight is complexed with a cyclodextrin. The actual percent of active agent that is complexed will vary according to the complexation equilibrium constant characterizing the complexation of a specific cyclodextrin to a specific active agent. The invention also includes embodiments wherein the active agent is not complexed with the cyclodextrin or wherein a minor portion of the active agent is complexed with the derivatized cyclodextrin. It should be noted that an SAE-CD, or any other anionic derivatized cyclodextrin, can form one or more ionic bonds with a positively charged compound. This ionic association can occur regardless of whether the positively

charged compound is complexed with the cyclodextrin either by inclusion in the cavity or formation of a salt bridge. The preservative property of SAE-CD does not require inclusion complexation or salt formation with an active agent.

The amount of derivatized cyclodextrin required to provide the desired level of preservation will vary according to the materials comprising the formulation. The more prone a formulation is to microbial contamination, the higher the amount of derivatized cyclodextrin that may be required. The less prone a formulation is to microbial contamination, the lower the amount of derivatized cyclodextrin that may be required. In the absence of other preservative, the formulation comprises greater than about 25 % w/v of derivatized cyclodextrin for biocidal effect or greater than about 4.8%±0.5% for biostasis.

The parent cyclodextrins have limited water solubility as compared to SAE-CD and HPCD. Underivatized α-CD has a water solubility of about 14.5% w/v at saturation. Underivatized β-CD has a water solubility of about 1.85% w/v at saturation. Underivatized γ-CD has a water solubility of about 23.2% w/v at saturation. At these concentrations, these parent cyclodextrins are unable to, on their own, preserve formulations containing them. Dimethyl-beta-cyclodextrin (DMCD) forms a 43% w/w aqueous solution at saturation. At this concentration, DMCD is unable to preserve a formulation containing it.

However, the present inventors have discovered that SAE-CD in addition to other cyclodextrin derivatives possesses the ability to reduce water activity in the formulations of the invention. It is believed that the formulation-preserving activity of the derivatized cyclodextrins is at least in part due to their ability to reduce water activity. Generally, the greater the water activity-reducing power of a derivatized cyclodextrin, the lower the amount of cyclodextrin required to preserve a solution. It is also possible that an ionized derivatized cyclodextrin possesses preservative activity when present as a salt. The preservative activity of a derivatized cyclodextrin might also be the result of its osmolality.

Other water soluble cyclodextrin derivatives that decrease water activity can be used according to the invention. Exemplary derivatives include the hydroxyethyl, hydroxypropyl (including 2- and 3-hydroxypropyl) and dihydroxypropyl ethers, their corresponding mixed ethers and further mixed ethers with methyl or ethyl groups, such as methylhydroxyethyl, ethyl-hydroxyethyl and ethyl-hydroxypropyl ethers of alpha-, beta-

and gamma-cyclodextrin; and the maltosyl, glucosyl and maltotriosyl derivatives of alpha , beta- and gamma-cyclodextrin, which may contain one or more sugar residues, e.g. glucosyl or diglucosyl, maltosyl or dimaltosyl, as well as various mixtures thereof, e.g. a mixture of maltosyl and dimaltosyl derivatives. Specific cyclodextrin derivatives for use herein include hydroxypropyl-beta-cyclodextrin, hydroxyethyl-beta-cyclodextrin, hydroxypropyl-gamma-cyclodextrin, hydroxyethyl-gamma-cyclodextrin, dihydroxypropyl-beta-cyclodextrin, glucosyl-alpha-cyclodextrin, glucosyl-beta-cyclodextrin, diglucosyl-beta-cyclodextrin, maltosyl-alpha-cyclodextrin, maltosyl-beta-cyclodextrin, maltosyl-gamma-cyclodextrin, maltotriosyl-beta-cyclodextrin, maltotriosyl-gamma-cyclodextrin and dimaltosyl-beta-cyclodextrin, and mixtures thereof such as maltosyl-beta-cyclodextrin/dimaltosyl-beta-cyclodextrin, as well as methyl-beta-cyclodextrin. Procedures for preparing such cyclodextrin derivatives are well-known, for example, from Bodor United States Patent No. 5,024,998 dated June 18, 1991, and references cited therein.

The HPCD can be obtained from Research Diagnostics Inc. (Flanders, NJ). HPCD is available with different degrees of substitution. Exemplary products include ENCAPSIN™ (degree of substitution~4; HP4-β-CD) and MOLECUSOL™ (degree of substitution~8; HP8-β-CD); however, embodiments including other degrees of substitution are also available. Since HPCD is non-ionic, it is not available in salt form. As with other derivatized cyclodextrins of the invention, changes in the degree of substitution can result in changes in the ability of the HPCD to reduce water activity and preserve a formulation.

Dimethyl cyclodextrin is available from FLUKA Chemie (Buchs, CH) or Wacker (Iowa). Other derivatized cyclodextrins suitable in the invention include water soluble derivatized cyclodextrins. Exemplary water-soluble derivatized cyclodextrins include carboxylated derivatives; sulfated derivatives; alkylated derivatives; hydroxyalkylated derivatives; methylated derivatives; and carboxy-β-cyclodextrins, e.g. succinyl-β-cyclodextrin (SCD), and 6^A-amino-6^A-deoxy-N-(3-carboxypropyl)-β-cyclodextrin. All of these materials can be made according to methods known in the prior art. Suitable derivatized cyclodextrins are disclosed in Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry (Eds. Christopher J. Easton, Stephen F. Lincoln, Imperial College Press, London, UK, 1999) and New Trends in Cyclodextrins and Derivatives (Ed. Dominique Duchene, Editions de Santé, Paris, France, 1991).

Sulfobutylether β-cyclodextrin (Captisol, CyDex Inc., degree of substitution = 6.6, lot no. CY03A129017), 2-hydroxypropyl β-cyclodextrin (HPBCD, Cerestar, degree of substitution = 5.5, lot no. 1Y0048), succinylated-β-cyclodextrin (S-CD, Cyclolab, batch no. CYL-1644), and 2,6,di-o-methyl-β-cyclodextrin (DM-CD, Fluka, lot no. 424522/1) %w/w solutions were prepared at their native pH. The water activity of the solutions were determined at ambient temperature and the results presented in Figure 4.

As the concentration of the cyclodextrin derivative is increased, the water activity decreases; however, the extent to which water activity decreases varies according to the agent used. Captisol is surprisingly more effective as a water activity-reducing agent than the other modified cyclodextrins. It should be noted that the water activity of the dimethyl cyclodextrin solution was determined at the saturation concentration of ~43%w/w. Captisol is also more effective at reducing the water activity compared to the underivatized cyclodextrins. The measured water activity of the alpha, beta, and gamma cyclodextrins at their saturation solubility is 0.980 (14.0%w/v), 0.982 (1.3%w/v), 0.978 (22.7%w/v), respectively.

The concentration of SAE-CD in solution can be expressed on a weight to weight or weight to volume basis; however, these two units are interconvertible. When a known weight of Captisol is dissolved in a known weight of water, the %w/w Captisol concentration is determined by dividing the Captisol weight in grams by the total weight (Captisol + water weight) in like units and multiplying by 100. When a known weight of Captisol is dissolved to a known total volume, the %w/v Captisol concentration is determined by dividing the Captisol weight in grams by the total volume in milliliters and multiplying by 100. The correlation between the two Captisol concentration percentages was experimentally determined by preparing various %w/w Captisol solutions and measuring the density of each with a pycnometer at 25°C. The density (g/mL) of each %w/w Captisol solution is presented in the table below.

Captisol % w/w	Density (g/mL)
59.4	1.320
49.4	1.259
39.7	1.202
29.8	1.149
19.7	1.095
8.5	1.041
0.0	1.002
slope =	0.0053
y-intercept =	0.995
correlation =	0.9989

The resulting linear relationship readily enables the conversion of Captisol concentrations expressed in %w/w to that of %w/v by the following equation:

$$\%w/v = [(\%w/w * slope) + y\text{-intercept}] * \%w/w$$

where the slope and intercept values are determined from a linear regression of the density data in the table.

For example, by using the above equation, a 40%w/w Captisol solution would be equivalent to a ~48.3% w/v Captisol solution.

The water activity values detailed herein are approximate and can vary from instrument to instrument. These values were determined according to the procedure described herein on a water activity meter described herein. The numbers can also vary within the standard deviation of a particular instrument. It is also possible for the numbers to vary according to the accuracy and reproducibility of the instrument used as well as the method for calibrating the instrument with solution standards of known water activity.

The standard deviation of reproducibility and accuracy can vary widely or narrowly depending upon the experimental conditions used to measure the water activity or upon operator skill. Typically, a standard deviation of ±0.02 is permissible; although lower and higher standard deviations may be observed.

The water activity of sulfobutyl ether β-cyclodextrin (SBECD) with varying average degrees of substitution (DS≈1, 7, 14) was determined for solutions of varying concentration. The results are presented in Figure 5 and indicate that, on a equivalent weight or molar basis, water activity is lower for SAE-CD with higher degrees of substitution, meaning that the water-activity reducing potential of SAE-CD increases as

the degree of substitution increases. Accordingly, a formulation containing SBE1-β-CD will generally require more cyclodextrin, on a weight or molar basis, than would a formulation containing SBE7-β-CD to obtain an equivalent reduction in water activity.

A water activity-reducing agent is a compound or mixture of compounds capable of reducing the water activity of the formulation. Increasing the concentration of a water activity-reducing agent in the formulation causes a decrease in the water activity of the formulation. Almost any material dissolved in an aqueous solution in sufficient amounts will decrease the water activity of the solution. However, suitable materials normally used for this purpose include PEG, glycol, polyol, glycerin, propylene glycol, propanediol, surfactant, detergent, soap, benzyl alcohol, sugar and other polysaccharides, salt and other electrolytes, thickening agent, hygroscopic agent, equilibrium protecting agent, deliquescent agent, hydrogenated glucose syrup (lycasin), mannitol, triacetin, tetraglycol, PVP, humectants, cellulose derivatives, gums such as guar, xanthan and gum arabic, and other materials known to those of skill in the art, and combinations thereof.

Poly(ethylene glycol), PEG, and poly(vinyl pyrrolidone), PVP, are known water activity reducing agents. In order to assess the effect that each copolymer has on the water activity of Captisol solutions, numerous %w/w solutions were prepared and analyzed. The determined water activity values are presented in Figures 6-8.

Based upon the results obtained, PEG and PVP of two different molecular weights, PVP-12 and PVP-17 all reduce the water activity of the Captisol solutions. Therefore, the water activity of a formulation can be reduced by a water soluble derivatized cyclodextrin or a combination of a water soluble derivatized cyclodextrin and one or more other components, such as a water activity-reducing agent.

Accordingly, the invention also provides a method of preserving a formulation by including a derivatized cyclodextrin in the formulation, wherein the derivatized cyclodextrin is included in an amount sufficient to reduce the water activity of the formulation an amount sufficient to provide a preserved formulation.

In one exemplary embodiment, a water activity reducing agent is included in a formulation containing a derivatized or non-derivatized cyclodextrin and neither the cyclodextrin nor the water activity-reducing agent is present in an amount sufficient to individually reduce the water activity to the desired value to preserve the formulation. In other words, the water activity-reducing material and cyclodextrin together can provide an

improved, additive or synergistic enhancement over the water activity-reducing effect of either material alone.

One or more water activity-reducing agents can be used in combination with one or more derivatized or non-derivatized cyclodextrins in the formulation.

Accordingly, the invention provides an alternate method of increasing the shelf-life of (such as by preserving) a formulation that sustains microbial growth. The formulation of the alternate method would comprise an aqueous carrier, and a first water activity-reducing agent present in an amount insufficient to, on its own, preserve the formulation. The method would comprise the step of including a derivatized or non-derivatized cyclodextrin in the formulation. By so doing, the first water activity-reducing agent and the cyclodextrin cooperate to improve the shelf-life of the formulation. This can be done even when the cyclodextrin is present in an amount insufficient to, on its own, preserve the formulation.

A formulation according to the invention will have a storage shelf life of no less than three days, one week, three weeks, one month, three months, six months, or one year. In this case, shelf life is determined only as regards the increase in bioburden in the formulation. For example, for a formulation having a shelf life of at least six months, the formulation will not demonstrate an unacceptable and substantial increase in bioburden during the storage period of at least six months. The criteria for acceptable shelf-life are set as needed according to a given product and its storage stability requirements. In other words, the bioburden of a formulation having an acceptable shelf-life will not increase beyond a predetermined value during the intended period of storage. On the other hand, the bioburden of a formulation having an unacceptable shelf-life will increase beyond the predetermined value during the intended period of storage. It should be noted that a shelf-life of as little as one week is suitable for products that are compounded by a pharmacist and sold to customers of a pharmacy. At a minimum, a preserved formulation will have an acceptable shelf-life of at least 24-hours after initial contamination. This is particularly true to for multi-dose parenteral formulations. In other embodiments, a preserved formulation will have a shelf-life of at least 28 days as per USP guidelines. In another embodiment, a preserved formulation will have a shelf-life of at least two years.

Osmolality is related to water activity and thus can also be an indicator of formulation preserving activity. Increases in osmolality correspond to decreases in water activity. Figure 3 depicts the relationship between osmolality and concentration of

SBE-CD in an unbuffered aqueous solution at room temperature. At concentrations up to about 20% to 30% w/v, the relationship is linear. At SBE-CD concentrations below about 11-13% w/v, the solutions are hypotonic or hypoosmotic with respect to blood and at SBE-CD concentrations above about 11-13% w/v the SBE-CD containing solutions are hypertonic or hyperosmotic with respect to blood. When red blood cells are exposed to solutions that are hypo- or hypertonic, they can shrink or swell in size which can lead to hemolysis. As noted above and in Figure 1, SBE-CD is less prone to induce hemolysis than other derivatized cyclodextrins.

A formulation containing the active agent propofol at 1% w/v and 22% w/v CAPTISOL is hypertonic and has an osmolality of 687 mOsmoles/kg. A lipid emulsion formulation of 1% propofol (the marketed product Diprivan) has a measure osmolality of 303 mOsmoles/kg and is isoosmotic with blood. The hemolytic potential of each formulation was evaluated according to Vasque et al. ("In Vitro Evaluation Of Biocompatibility Of A Dose Formulation" presented at the 40th Annual Meeting of the Society of Toxicology, San Francisco, CA, (Mar 25-29, 2001)). The data indicated there was no measured evidence for hemolysis caused by the Captisol formulation at any of the formulation:blood ratios. The Diprivan formulation induced moderate hemolysis at formulation:blood ratios of 1:1 and slight hemolysis at ratios of 0.5:1, 0.25:1 and 0.1:1.

Accordingly, the invention also provides a liquid formulation of an active agent and an SAE-CD, wherein the formulation has a reduced hemolytic potential as compared to other cyclodextrin-based formulations.

The formulation can include one or more of any known active agents. The active agent included in the present invention can possess a wide range of values for water solubility, bioavailability and hydrophilicity. Active agents to which the present invention is particularly suitable include water insoluble, poorly water soluble, slightly water soluble, moderately water soluble, water soluble, very water soluble, hydrophobic, or hydrophilic therapeutic agents. It will be understood by the artisan of ordinary skill that an active agent used in the formulation of the present invention is independently selected at each occurrence from any known active agent and from those disclosed herein. It is not necessary that the active agent complex with the derivatized cyclodextrin or form an ionic association with the derivatized cyclodextrin.

Active agents generally include physiologically or pharmacologically active substances that produce a systemic or localized effect or effects on animals and human

beings. Active agents also include pesticides, herbicides, insecticides, antioxidants, plant growth instigators, sterilization agents, catalysts, chemical reagents, food products, nutrients, cosmetics, vitamins, sterility inhibitors, fertility instigators, microorganisms, flavoring agents, sweeteners, cleansing agents and other such compounds for pharmaceutical, veterinary, horticultural, household, food, culinary, agricultural, cosmetic, industrial, cleaning, confectionery and flavoring applications. The active agent can be present in its neutral, ionic, salt, basic, acidic, natural, synthetic, diastereomeric, isomeric, enantiomerically pure, racemic, hydrate, chelate, derivative, analog, or other common form.

The formulation of the invention can be used to deliver two or more different active agents. Particular combinations of active agents can be provided by the present capsule. Some combinations of active agents include: 1) a first drug from a first therapeutic class and a different second drug from the same therapeutic class; 2) a first drug from a first therapeutic class and a different second drug from a different therapeutic class; 3) a first drug having a first type of biological activity and a different second drug having about the same biological activity; 4) a first drug having a first type of biological activity and a different second drug having a different second type of biological activity. Exemplary combinations of active agents are described herein.

Representative active agents include nutrients and nutritional agents, hematological agents, endocrine and metabolic agents, cardiovascular agents, renal and genitourinary agents, respiratory agents, central nervous system agents, gastrointestinal agents, anti-infective agents, biologic and immunological agents, dermatological agents, ophthalmic agents, antineoplastic agents, and diagnostic agents. Exemplary nutrients and nutritional agents include as minerals, trace elements, amino acids, lipotropic agents, enzymes and chelating agents. Exemplary hematological agents include hematopoietic agents, antiplatelet agents, anticoagulants, coumarin and indandione derivatives, coagulants, thrombolytic agents, antisickling agents, hemorrheologic agents, antihemophilic agents, hemostatics, plasma expanders and hemin. Exemplary endocrine and metabolic agents include sex hormones, uterine-active agents, bisphosphonates, antidiabetic agents, glucose elevating agents, adrenocortical steroids, parathyroid hormone, thyroid drugs, growth hormones, posterior pituitary hormones, octreotide acetate, imiglucerase, calcitonin-salmon, sodium phenylbutyrate, betaine anhydrous, cysteamine bitartrate, sodium

benzoate and sodium phenylacetate, bromocriptine mesylate, cabergoline, agents for gout, and antidotes.

Exemplary cardiovascular agents include nootropic agents, antiarrhythmic agents, calcium channel blocking agents, vasodilators, antiadrenergics/sympatholytics, renin angiotensin system antagonists, antihypertensive combinations, agents for pheochromocytoma, agents for hypertensive emergencies, antihyperlipidemic agents, antihyperlipidemic combination products, vasopressors used in shock, potassium removing resins, edetate disodium, cardioplegic solutions, agents for patent ductus arteriosus, and sclerosing agents. Exemplary renal and genitourinary agents include interstitial cystitis agents, cellulose sodium phosphate, anti-impotence agents, acetohydroxamic acid (aha), genitourinary irrigants, cystine-depleting agents, urinary alkalinizers, urinary acidifiers, anticholinergics, urinary cholinergics, polymeric phosphate binders, vaginal preparations, and diuretics. Exemplary respiratory agents include bronchodilators, leukotriene receptor antagonists, leukotriene formation inhibitors, respiratory inhalant products, nasal decongestants, respiratory enzymes, lung surfactants, antihistamines, nonnarcotic antitussives, and expectorants. Exemplary central nervous system agents include CNS stimulants, narcotic agonist analgesics, narcotic agonist-antagonist analgesics, central analgesics, acetaminophen, salicylates, nonnarcotic analgesics, nonsteroidal anti-inflammatory agents, agents for migraine, antiemetic/antivertigo agents, antianxiety agents, antidepressants, antipsychotic agents, cholinesterase inhibitors, nonbarbiturate sedatives and hypnotics, nonprescription sleep aids, barbiturate sedatives and hypnotics, general anesthetics, injectable local anesthetics, anticonvulsants, muscle relaxants, antiparkinson agents, adenosine phosphate, cholinergic muscle stimulants, disulfuram, smoking deterrents, riluzole, hyaluronic acid derivatives, and botulinum toxins. Exemplary gastrointestinal agents including H pylori agents, histamine H2 antagonists, proton pump inhibitors, sucralfate, prostaglandins, antacids, gastrointestinal anticholinergics/antispasmodics, mesalamine, olsalazine sodium, balsalazide disodium, sulfasalazine, celecoxib, infliximab, tegaserod maleate, laxatives, antidiarrheals, antiflatulents, lipase inhibitors, GI stimulants, digestive enzymes, gastric acidifiers, hydrocholeretics, gallstone solubilizing agents, mouth and throat products, systemic deodorizers, and anorectal preparations. Exemplary anti-infective agents including penicillins, cephalosporins and related antibiotics, carbapenem, monobactams, chloramphenicol, quinolones, fluoroquinolones, tetracyclines, macrolides, spectinomycin,

streptogramins, vancomycin, oxalodinones, lincosamides, oral and parenteral aminoglycosides, colistimethate sodium, polymyxin b sulfate, bacitracin, metronidazole, sulfonamides, nitrofurans, methenamines, folate antagonists, antifungal agents, antimalarial preparations, antituberculosis agents, amebicides, antiviral agents, antiretroviral agents, leprostatics, antiprotozoals, anthelmintics, and cdc anti-infective agents. Exemplary biologic and immunological agents including immune globulins, monoclonal antibody agents, antivenins, agents for active immunization, allergenic extracts, immunologic agents, and antirheumatic agents. Exemplary dermatological agents includw topical antihistamine preparations, topical anti-infectives, anti-inflammatory agents, anti-psoriatic agents, antiseborrheic products, arnica, astringents, cleansers, capsaicin, destructive agents, drying agents, enzyme preparations, topical immunomodulators, keratolytic agents, liver derivative complex, topical local anesthetics, minoxidil, eflornithine HCl, photochemotherapy agents, pigment agents, topical poison ivy products, topical pyrimidine antagonist, pyrithione zinc, retinoids, rexinoids, scabicides/pediculicides, wound healing agents, emollients, protectants, sunscreens, ointment and lotion bases, rubs and liniments, dressings and granules, and physiological irrigating solutions. Exemplary ophthalmic agents include agents for glaucoma, mast cell stabilizers, ophthalmic antiseptics, ophthalmic phototherapy agents, ocular lubricants, artificial tears, ophthalmic hyperosmolar preparations, and contact lens products. Exempalry antineoplastic agents include alkylating agents, antimetabolites, antimitotic agents, epipodophyllotoxins, antibiotics, hormones, enzymes, radiopharmaceuticals, platinum coordination complex, anthracenedione, substituted ureas, methylhydrazine derivatives, imidazotetrazine derivatives, cytoprotective agents, dna topoisomerase inhibitors, biological response modifiers, retinoids, rexinoids, monoclonal antibodies, protein-tyrosine kinase inhibitors, porfimer sodium, mitotane (o, p'-ddd), and arsenic trioxide. Exemplary diagnostic agents include in vivo diagnostic aids, in vivo diagnostic biologicals, and radiopaque agents.

The above-mentioned list should not be considered exhaustive and is merely exemplary of the many embodiments considered within the scope of the invention. Many other active agents can be administered with the formulation of the present invention.

An active agent contained within the present formulation can be present as its pharmaceutically acceptable salt. As used herein, "pharmaceutically acceptable salt" refers to derivatives of the disclosed compounds wherein the active agent is modified by

reacting it with an acid or base as needed to form an ionically bound pair. Examples of pharmaceutically acceptable salts include conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Suitable non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfonic, sulfamic, phosphoric, nitric and others known to those of ordinary skill in the art. The salts prepared from organic acids such as amino acids, acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and others known to those of ordinary skill in the art. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent active agent which contains a basic or acidic moiety by conventional chemical methods. Lists of other suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th. ed., Mack Publishing Company, Easton, PA, 1985, the relevant disclosure of which is hereby incorporated by reference.

An anionic derivatized cyclodextrin can complex or otherwise bind with an acid-ionizable agent. As used herein, the term acid-ionizable agent is taken to mean any compound that becomes or is ionized in the presence of an acid. An acid-ionizable agent comprises at least one acid-ionizable functional group that becomes ionized when exposed to acid or when placed in an acidic medium. Exemplary acid-ionizable functional groups include a primary amine, secondary amine, tertiary amine, quaternary amine, aromatic amine, unsaturated amine, primary thiol, secondary thiol, sulfonium, hydroxyl, enol and others known to those of ordinary skill in the chemical arts.

The degree to which an acid-ionizable agent is bound by non-covalent ionic binding versus inclusion complexation formation can be determined spectrophotometrically using methods such as ^{1}HNMR, ^{13}CNMR, or circular dichroism, for example, and by analysis of the phase solubility data for the acid-ionizable agent and anionic derivatized cyclodextrin. The artisan of ordinary skill in the art will be able to use these conventional methods to approximate the amount of each type of binding that is occurring in solution to determine whether or not binding between the species is occurring predominantly by non-covalent ionic binding or inclusion complex formation. An acid-ionizable agent that binds to derivatized cyclodextrin by both means will generally exhibit a bi-phasic phase solubility curve. Under conditions where non-covalent ionic bonding

predominates over inclusion complex formation, the amount of inclusion complex formation, measured by NMR or circular dichroism, will be reduced even though the phase solubility data indicates significant binding between the species under those conditions; moreover, the intrinsic solubility of the acid-ionizable agent, as determined from the phase solubility data, will generally be higher than expected under those conditions.

As used herein, the term non-covalent ionic bond refers to a bond formed between an anionic species and a cationic species. The bond is non-covalent such that the two species together form a salt or ion pair. An anionic derivatized cyclodextrin provides the anionic species of the ion pair and the acid-ionizable agent provides the cationic species of the ion pair. Since an anionic derivatized cyclodextrin is multi-valent, an SAE-CD can form an ion pair with one or more acid-ionizable agents.

A liquid formulation of the invention may also be converted to a solid formulation for reconstitution. A reconstitutable solid pharmaceutical composition according to the invention comprises an active agent, a derivatized cyclodextrin and optionally at least one other pharmaceutical excipient. This composition is reconstituted with an aqueous liquid to form a liquid formulation that is preserved. The composition can comprise an admixture of a solid derivatized cyclodextrin and an active agent-containing solid and optionally at least one solid pharmaceutical excipient, such that a major portion of the active agent is not complexed with the derivatized cyclodextrin prior to reconstitution. Alternatively, the composition can comprise a solid mixture of a derivatized cyclodextrin and an active agent, wherein a major portion of the active agent is complexed with the derivatized cyclodextrin prior to reconstitution.

The reconstitutable formulation can be prepared according to any of the following processes. A liquid formulation of the invention is first prepared, then a solid is formed by lyophilization (freeze-drying), spray-drying, spray freeze-drying, antisolvent precipitation, various processes utilizing supercritical or near supercritical fluids, or other methods known to those of ordinary skill in the art to make a solid for reconstitution.

A liquid vehicle included in a formulation of the invention comprises an aqueous liquid carrier, such as water, aqueous alcohol, or aqueous organic solvent, or a non-aqueous liquid carrier.

Although not necessary, the formulation of the present invention may include a conventional preservative, antioxidant, buffering agent, acidifying agent, alkalizing agent,

colorant, solubility-enhancing agent, complexation enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavors, sweeteners, other excipients known by those of ordinary skill in the art for use in preserved formulations, or a combination thereof.

As used herein, the term "alkalizing agent" is intended to mean a compound used to provide alkaline medium for product stability. Such compounds include, by way of example and without limitation, ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium bicarbonate, sodium hydroxide, triethanolamine, diethanolamine, organic amine base, alkaline amino acids and trolamine and others known to those of ordinary skill in the art.

As used herein, the term "acidifying agent" is intended to mean a compound used to provide an acidic medium for product stability. Such compounds include, by way of example and without limitation, acetic acid, acidic amino acids, citric acid, fumaric acid and other alpha hydroxy acids, hydrochloric acid, ascorbic acid, phosphoric acid, sulfuric acid, tartaric acid and nitric acid and others known to those of ordinary skill in the art.

As used herein, a conventional preservative is a compound used to at least reduce the rate at which bioburden increases, but preferably maintains bioburden steady or reduces bioburden after contamination. Such compounds include, by way of example and without limitation, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, phenylmercuric acetate, thimerosal, metacresol, myristylgamma picolinium chloride, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, sorbic acid, thymol, and methyl, ethyl, propyl or butyl parabens and others known to those of ordinary skill in the art.

As used herein, the term "antioxidant" is intended to mean an agent that inhibits oxidation and thus is used to prevent the deterioration of preparations by the oxidative process. Such compounds include, by way of example and without limitation, acetone, potassium metabisulfite, potassium sulfite, ascorbic acid, ascorbyl palmitate, citric acid, butylated hydroxyanisole, butylated hydroxytoluene, hypophophorous acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium citrate, sodium sulfide, sodium sulfite, sodium bisulfite, sodium formaldehyde sulfoxylate, thioglycolic acid, EDTA, pentetate, and sodium metabisulfite and others known to those of ordinary skill in the art.

As used herein, the term "buffering agent" is intended to mean a compound used to resist change in pH upon dilution or addition of acid or alkali. Such compounds include, by way of example and without limitation, acetic acid, sodium acetate, adipic acid, benzoic acid, sodium benzoate, boric acid, sodium borate, citric acid, glycine, maleic acid, monobasic sodium phosphate, dibasic sodium phosphate, HEPES, lactic acid, tartaric acid, potassium metaphosphate, potassium phosphate, monobasic sodium acetate, sodium bicarbonate, tris, sodium tartrate and sodium citrate anhydrous and dihydrate and others known to those of ordinary skill in the art.

A complexation-enhancing agent can be added to a formulation of the invention. When such an agent is present, the ratio of cyclodextrin /active agent can be changed. A complexation-enhancing agent is a compound, or compounds, that enhance(s) the complexation of the active agent with the cyclodextrin. Suitable complexation enhancing agents include one or more pharmacologically inert water soluble polymers, hydroxy acids, and other organic compounds typically used in preserved formulations to enhance the complexation of a particular agent with cyclodextrins.

Hydrophilic polymers can be used as complexation-enhancing, solubility-enhancing and/or water activity reducing agents to improve the performance of formulations containing a cyclodextrin-based preservative. Loftsson has disclosed a number of polymers suitable for combined use with a cyclodextrin (underivatized or derivatized) to enhance the performance and/or properties of the cyclodextrin. Suitable polymers are disclosed in *Pharmazie* (2001), 56(9), 746-747; *International Journal of Pharmaceutics* (2001), 212(1), 29-40; Cyclodextrin: From Basic Research to Market, International Cyclodextrin Symposium, 10th, Ann Arbor, MI, United States, May 21-24, 2000 (2000), 10-15 (Wacker Biochem Corp.: Adrian, Mich.); PCT International Publication No. WO 9942111; *Pharmazie*, 53(11), 733-740 (1998); *Pharm. Technol. Eur.*, 9(5), 26-34 (1997); *J. Pharm. Sci.* 85(10), 1017-1025 (1996); European Patent Application EP0579435; Proceedings of the International Symposium on Cyclodextrins, 9th, Santiago de Comostela, Spain, May 31-June 3, 1998 (1999), 261-264 (Editor(s): Labandeira, J. J. Torres; Vila-Jato, J. L. Kluwer Academic Publishers, Dordrecht, Neth); *S.T.P. Pharma Sciences* (1999), 9(3), 237-242; ACS Symposium Series (1999), 737(Polysaccharide Applications), 24-45; *Pharmaceutical Research* (1998), 15(11), 1696-1701; *Drug Development and Industrial Pharmacy* (1998), 24(4), 365-370; *International Journal of Pharmaceutics* (1998), 163(1-2), 115-121; Book of Abstracts, 216th ACS National

Meeting, Boston, August 23-27 (1998), CELL-016, American Chemical Society; *Journal of Controlled Release*, (1997), 44/1 (95-99); *Pharm.Res.* (1997) 14(11), S203; *Investigative Ophthalmology & Visual Science*, (1996), 37(6), 1199-1203; Proceedings of the International Symposium on Controlled Release of Bioactive Materials (1996), 23rd, 453-454; *Drug Development and Industrial Pharmacy* (1996), 22(5), 401-405; Proceedings of the International Symposium on Cyclodextrins, 8th, Budapest, Mar. 31-Apr. 2, (1996), 373-376. (Editor(s): Szejtli, J.; Szente, L. Kluwer: Dordrecht, Neth.); *Pharmaceutical Sciences* (1996), 2(6), 277-279; *European Journal of Pharmaceutical Sciences*, (1996) 4(SUPPL.), S144; Third European Congress of Pharmaceutical Sciences Edinburgh, Scotland, UK September 15-17, 1996; *Pharmazie*, (1996), 51(1), 39-42; *Eur. J. Pharm. Sci.* (1996), 4(Suppl.), S143; U.S. Patents No. 5,472,954 and No. 5,324,718; *International Journal of Pharmaceutics* (Netherlands), (Dec. 29, 1995) 126, 73-78; Abstracts of Papers of the American Chemical Society, (02 APR 1995) 209(1), 33-CELL; *European Journal of Pharmaceutical Sciences*, (1994) 2, 297-301; *Pharmaceutical Research* (New York), (1994) 11(10), S225; *International Journal of Pharmaceutics* (Netherlands), (Apr 11, 1994) 104, 181-184; and *International Journal of Pharmaceutics* (1994), 110(2), 169-77, the entire disclosures of which are hereby incorporated by reference.

Other suitable polymers are well-known excipients commonly used in the field of pharmaceutical formulations and are included in, for example, *Remington's Pharmaceutical Sciences, 18th Edition*, Alfonso R. Gennaro (editor), Mack Publishing Company, Easton, PA, 1990, pp. 291-294; Alfred Martin, James Swarbrick and Arthur Commarata, *Physical Pharmacy. Physical Chemical Principles in Pharmaceutical Sciences, 3rd edition* (Lea & Febinger, Philadelphia, PA, 1983, pp. 592-638); A.T. Florence and D. Altwood, (*Physicochemical Principles of Pharmacy, 2nd Edition*, MacMillan Press, London, 1988, pp. 281-334. The entire disclosures of the references cited herein are hereby incorporated by references. Still other suitable polymers include water-soluble natural polymers, water-soluble semi-synthetic polymers (such as the water-soluble derivatives of cellulose) and water-soluble synthetic polymers. The natural polymers include polysaccharides such as inulin, pectin, algin derivatives (e.g. sodium alginate) and agar, and polypeptides such as casein and gelatin. The semi-synthetic polymers include cellulose derivatives such as methylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, their mixed ethers such as hydroxypropyl methylcellulose and

other mixed ethers such as hydroxyethyl ethylcellulose and hydroxypropyl ethylcellulose, hydroxypropyl methylcellulose phthalate and carboxymethylcellulose and its salts, especially sodium carboxymethylcellulose. The synthetic polymers include polyoxyethylene derivatives (polyethylene glycols) and polyvinyl derivatives (polyvinyl alcohol, polyvinylpyrrolidone and polystyrene sulfonate) and various copolymers of acrylic acid (e.g. carbomer). Other natural, semi-synthetic and synthetic polymers not named here which meet the criteria of water solubility, pharmaceutical acceptability and pharmacological inactivity are likewise considered to be within the ambit of the present invention.

When present, active agents may or may not bind or complex with the cyclodextrin. It is only necessary that the cyclodextrin be present in the formulation in an amount sufficient to preserve the formulation upon storage thereof for a predetermined time.

As used herein, the term "stabilizer" is intended to mean a compound used to stabilize the therapeutic agent against physical, chemical, or biochemical process which would reduce the therapeutic activity of the agent. Suitable stabilizers include, by way of example and without limitation, albumin, sialic acid, creatinine, glycine and other amino acids, niacinamide, sodium acetyltryptophonate, zinc oxide, sucrose, glucose, lactose, sorbitol, mannitol, glycerol, polyethylene glycols, sodium caprylate and sodium saccharin and other known to those of ordinary skill in the art.

As used herein, the term "tonicity modifier" is intended to mean a compound or compounds that can be used to adjust the tonicity of the liquid formulation. Suitable tonicity modifiers include glycerin, lactose, mannitol, dextrose, sodium chloride, sodium sulfate, sorbitol, trehalose and others known to those of ordinary skill in the art. In one embodiment, the tonicity of the liquid formulation approximates the tonicity of blood or plasma.

As used herein, the term "antifoaming agent" is intended to mean a compound or compounds that prevents or reduces the amount of foaming that forms on the surface of the liquid formulation. Suitable antifoaming agents include dimethicone, simethicone, octoxynol and others known to those of ordinary skill in the art.

As used herein, the term "bulking agent" is intended to mean a compound used to add bulk to the lyophilized product and/or assist in the control of the properties of the formulation during lyophilization. Such compounds include, by way of example and

without limitation, dextran, trehalose, sucrose, polyvinylpyrrolidone, lactose, inositol, sorbitol, dimethylsulfoxide, glycerol, albumin, calcium lactobionate, and others known to those of ordinary skill in the art.

As used herein, the term "cryoprotectant" is intended to mean a compound used to protect an active therapeutic agent from physical or chemical degradation during lyophilization. Such compounds include, by way of example and without limitation, dimethyl sulfoxide, glycerol, trehalose, propylene glycol, polyethylene glycol, and others known to those of ordinary skill in the art.

As used herein, the term "emulsifier" or "emulsifying agent" is intended to mean a compound added to one or more of the phase components of an emulsion for the purpose of stabilizing the droplets of the internal phase within the external phase. Such compounds include, by way of example and without limitation, lecithin, polyoxylethylene-polyoxypropylene ethers, polyoxylethylene-sorbitan monolaurate, polysorbates, sorbitan esters, stearyl alcohol, tyloxapol, tragacanth, xanthan gum, acacia, agar, alginic acid, sodium alginate, bentonite, carbomer, carboxymethyl cellulose sodium, cholesterol, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, octoxynol, oleyl alcohol, polyvinyl alcohol, povidone, propylene glycol monostearate, sodium lauryl sulfate, and others known to those of ordinary skill in the art.

A solubility-enhancing agent can be added to the formulation of the invention. A solubility-enhancing agent is a compound, or compounds, that enhance(s) the solubility of the active agent when in a liquid formulation. When such an agent is present, the ratio of cyclodextrin/active agent can be changed. Suitable solubility enhancing agents include one or more organic solvents, detergents, soaps, surfactant and other organic compounds typically used in parenteral formulations to enhance the solubility of a particular agent.

Suitable organic solvents include, for example, ethanol, glycerin, polyethylene glycols, propylene glycol, poloxomers, and others known to those of ordinary skill in the art.

The formulation of the invention can also include oils, for example, fixed oils, such as peanut oil, sesame oil, cottonseed oil, corn oil and olive oil; fatty acids, such as oleic acid, stearic acid and isostearic acid; and fatty acid esters, such as ethyl oleate, isopropyl myristate, fatty acid glycerides and acetylated fatty acid glycerides. It can also include alcohols, such as ethanol, isopropanol, hexadecyl alcohol, glycerol and propylene glycol;

glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol; ethers, such as poly(ethylene glycol) 450; with petroleum hydrocarbons, such as mineral oil and petrolatum; water; or with mixtures thereof; with or without the addition of a pharmaceutically suitable surfactant, suspending agent or emulsifying agent.

It should be understood, that compounds used in the art of pharmaceutical formulations generally serve a variety of functions or purposes. Thus, if a compound named herein is mentioned only once or is used to define more than one term herein, its purpose or function should not be construed as being limited solely to that named purpose(s) or function(s).

The formulation of the invention can also include biological salt(s), sodium chloride, potassium chloride, or other electrolyte(s).

Since some active agents are subject to oxidative degradation, a liquid formulation according to the invention can have its oxygen removed. For example, the headspace of the container with the liquid formulation is made oxygen free, substantially oxygen free, or oxygen-reduced by purging the headspace with an inert gas, such as nitrogen or argon, or by bubbling the inert gas through the liquid formulation. For long-term storage, the liquid formulation containing an active agent subject to oxidative degradation is preferably stored in an oxygen-free or oxygen-reduced environment. Removal of oxygen from the formulation will enhance preservation of the formulation against aerobic microbes; whereas, addition of oxygen to the formulation will enhance preservation against anaerobic microbes.

The cyclodextrin-based formulations demonstrate reduced support for microbial growth as compared to the corresponding lipid emulsion formulations. Example 3 details the results of side-by-side comparisons of three different SAE-CD containing solutions, the lipid emulsion containing DIPRIVAN® and Baxter's Propofol Injectable Emulsion formulations in terms of their ability to sustain microbial growth. Each of the SAE-CD containing solutions contained propofol (1% wt.) and SBE7-β-CD (22% w/v). In addition, one of the three solutions contained disodium EDTA (0.005% w/v) at pH 8.2 to mimic the DIPRIVAN® formulation, and another of the three solutions contained sodium metabisulfite (0.025% w/v) at pH 5.5 to mimic the Baxter formulation. The third solution contained no added conventional preservative *per se*. The data (Tables 1-14 in Example 3) indicate that SAE-CD at the concentration tested does not sustain microbial growth. The effectiveness of SAE-CD was dependent upon the microbe on which it was tested;

however, in none of the samples of Example 3 was an increase in bioburden observed for solutions containing SAE-CD. Overall, the SAE-CD containing formulations actually had a lower microbial count after 24 and 48 hours than they did at the beginning of the study. Accordingly, the present formulation provides a substantially preserved parenteral formulation comprising an SAE-CD, an active agent and a liquid carrier. The invention also provides a method of preserving a formulation comprising the step of including in the formulation SAE-CD in an amount sufficient to slow down the rate of bioburden increase, maintain bioburden and/or reduce bioburden in a contaminated formulation. Unlike the emulsion formulation, the present formulation or composition does not require an added conventional preservative, such as detailed herein; although, a conventional preservative can be further included if desired. As such, the presently claimed formulations unexpectedly possess microbial growth retarding or preservative properties.

Additional embodiments of preserved formulations according to the invention are included in Examples 6-9. Formulations containing varying amounts of SBE7-β-CD and microbial inoculates were prepared. The microbes tested include *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella chloerasuis* (ATCC 13311), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404). As noted in the water activity versus microorganism table included in the Background section above, the above-mentioned microbes will not reproduce when the water activity of the medium in which they are found is at or below the values indicated. In other words, the microbes should proliferate when they are present in a medium having a water activity higher than the indicated values as long as the medium contains sufficient nutrients to sustain microbial growth. In order to provide a basis for unexpected results, the water activity values of the above-mentioned solutions were determined to be as follows:

CD Solution	Neat	Bound Active		Unbound Active
		10 mg/mL Propofol	20 mg/mL Ibuprofen	10 mg/mL Cyclopentolate HCl
20% w/v Captisol	0.974 (pH 5.5)	0.974 (pH 5.6)	Not Available	Not Available
40% w/v Captisol	0.951 (pH 6.0)	Not Available	0.979 (pH 5.7)	0.946 (pH 6.0)
40% w/v HPBCD	0.986 (pH 5.4)	Not Available	0.983 (pH 5.7)	0.986 (pH 6.0)

The antimicrobial preserving capability of the formulations was evaluated by testing each formulation using the procedure of USP XXV <51> test for preservative

effectiveness except that the time points analyzed were 0, 24, and 72 hours post inoculation.

In Example 6, the performance of SAE-CD to HPCD was evaluated in the presence and absence (control samples) of ibuprofen, a non-steroidal anti-inflammatory drug. Ibuprofen is known to complex with SAE-CD and HPCD. As detailed in the table above, complexation of SAE-CD with ibuprofen increased the a_w of the solution, but complexation of ibuprofen with HPCD did not increase the water activity of the HPCD-containing solution. Even so, the results indicate that a derivatized cyclodextrin, such as an SAE-CD, acts as a preservative under conditions where one would not expect it to, since the SAE-CD inhibited or retarded growth and/or kill microbes at concentrations for HPCD that are inactive. Moreover, SAE-CD retained preservative activity at water activity values in which the microbes would have been expected to proliferate. For example, SAE-CD present at a concentration of 40.0% w/v in the presence of the active ibuprofen had a measured a_w of 0.979. At that a_w value, one would not expect that a water activity-reducing agent, such as SAE-CD, would have a preservative effect. However, the inventors have discovered that SAE-CD, but not HPCD, causes a decrease in bioburden (possesses biocidal activity) at that water activity value for *P. aeruginosa*, *E. coli*, *S. chloerasuis* and *C. albicans*.

According to the above, SAE-CD unexpectedly provides preservative properties, for solutions containing it, at water activity values that one would not expect to see such a high degree of activity.

In the formulation of Example 7, cyclopentolate HCl was used as it was determined not to complex to any significant extent with SAE-CD or HPCD, since the drug is very water soluble. It is possible that minor complexation of the cyclopentolate with cyclodextrin could occur under certain circumstances. As noted above, addition of cyclopentolate to a solution containing SAE-CD or HPCD did not substantially increase or decrease the water activity of the respective solution. SAE-CD possesses better biocidal/biostatic properties against *P. aeruginosa*, *E. coli*, and *S. chloerasuis* than HPCD.

The pH at which SAE-CD expresses its preservative activity is not limited to alkaline pH values. As noted herein, SAE-CD, when present in sufficient amounts, serves as a suitable preservative for solutions having a pH from 0-14, or an acidic pH, alkaline pH, or physiological pH.

SAE-CD can also be used to "unmask" or "reveal" the hidden preservative properties of active agents. Propofol is not known to possess any preservative properties on its own. In fact, the commercial emulsion formulation includes a conventional preservative to provide an acceptable shelf-life for the product. For instance, the currently-marketed Diprivan 10 mg/mL propofol product is formulated as an emulsion and is notorious for microbial growth. Example 8 describes the results obtained from evaluating the an aqueous formulation comprising SAE-CD and propofol. A self-preserving formulation can be prepared by mixing a known amount of water, inactive ingredients if necessary, cyclodextrin, and an active ingredient(s). In Example 8, the SAE-CD concentration is generally not sufficiently high to possess antimicrobial activity, and the active ingredient propofol is not known to possess any inherent antimicrobial activity. One would expect therefore, that the formulation would not be preserved when inoculated with a microbe. However, a 10 mg/mL propofol solution formulated in 20% w/v Captisol has been shown to be self-preserving. The results of Example 8 demonstrate that the SAE-CD is cooperating with the propofol to provide a preserved solution. Without being held bound to a particular mechanism, it is believed that the SAE-CD unmasks propofol antimicrobial properties. The self-preserving properties of the solution are related to the nature of the active compound, propofol, and not to that of the 20% w/v Captisol solution alone. Accordingly, the invention provides a method of unmasking the preservative activity (biocidal, growth retarding and/or biostatic activity) of a drug that is otherwise not known to possess such activity. In a specific embodiment, the drug is a phenolic (phenol based) drug.

As used herein, the term "biostatic" refers to the property of being able to maintain a substantially constant bioburden in a formulation over a predetermined period of time. A biostatic agent does not significantly reduce the bioburden in a formulation. In other words, an SAE-CD is said to be a biostatic agent if it can maintain the number of microbes in a formulation substantially constant during storage of the formulation, i.e. within about ± 20% of the bioburden when the formulation was first made and sealed and optionally sterilized. In terms of log reduction of bioburden, a biostatic effect is observed when the bioburden does not change by more than ±0.5 log reduction. A biostatic agent can maintain a substantially constant bioburden by inhibiting or slowing down cell proliferation/reproduction/multiplication and/or maintaining a substantially constant rate

of cell turnover. The term "bioburden" is taken to mean number of microbes present in a formulation.

As used herein, the term "biocidal" or "microbicidal" refers to the property of being able to kill a microbe. A biocidal agent can reduce the bioburden in a formulation by killing microbes therein. A biocidal agent need not lyse a microbe, as it can kill the microbe by any known mechanism. In terms of log reduction of bioburden, a biocidal effect is observed when the bioburden decreases by more than >/= 0.5 log reduction. If the rate of microbial reproduction approximates the rate of microbial death in a formulation, a biocidal agent may appear to act essentially as a biostatic agent, since the bioburden would necessarily be substantially constant. If the rate of microbial reproduction is slower than the rate of microbial death in a formulation, a biocidal agent will cause an overall decrease in the bioburden. An agent might be considered a biocidal agent if it slows the rate of proliferation to a rate that is slower than the rate of cell death. In this case, the bioburden will ultimately decrease with increasing storage time.

As used herein, the term "growth-retarding" refers to the property of being able to slow down the rate of proliferation, reproduction, or multiplication of a microbe. A growth-retarding agent may permit an increase in bioburden during storage of the formulation; however, the rate of increase in bioburden will be lower in the presence of the growth retarding agent than in its absence. A growth-retarding agent might also cause a decrease in bioburden during storage of a formulation. The decrease might be caused by a slowing of microbial reproduction in combination with a microbial death rate that exceeds the rate of reproduction.

The preservative property (biostatic, biocidal and/or growth retarding activity) of SAE-CD is time, concentration and microbe dependent. Example 9 details the results of some screening experiments wherein the concentration of SAE-CD was varied in sample solutions inoculated with different microbes. For *S. aureus, P. aeruginosa* and *E. coli*, a concentration of 30% w/v SAE-CD is sufficient to provide a biocidal preservative effect at 24, 48 and 72 hours after inoculation. For *C. albicans*, and *A. niger*, a concentration of 4.8 %w/v or higher is sufficient to provide a microbistatic preservative effect. And for S. choleraesuis a concentration of 49.3% w/v shows microbicidal preservative effect at 24, 48 and 72 hours.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of

sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term "patient" or "subject" are taken to mean warm blooded animals such as mammals, for example, cats, dogs, mice, guinea pigs, horses, bovine cows, sheep and humans.

A formulation of the invention will comprise an active agent present in an effective amount. By the term "effective amount", is meant the amount or quantity of active agent that is sufficient to elicit the required or desired response, or in other words, the amount that is sufficient to elicit an appreciable biological response when administered to a subject.

In view of the above description and the examples below, one of ordinary skill in the art will be able to practice the invention as claimed without undue experimentation. The foregoing will be better understood with reference to the following examples that detail certain procedures for the preparation of formulations according to the present invention. All references made to these examples are for the purposes of illustration. The following examples should not be considered exhaustive, but merely illustrative of only a few of the many embodiments contemplated by the present invention.

EXAMPLE 1

Exemplary formulations according to the invention were made according to the following general procedure. CAPTISOL® cyclodextrin was dissolved in water to form a solution containing about 220 mg/mL of CAPTISOL® cyclodextrin. Propofol was added to the SAE-CD containing solution until a concentration of about 10 mg/mL propofol was reached. An additional preservative was added and the pH adjusted with sodium hydroxide/hydrochloric acid as indicated in the table below.

	Amount		
Ingredient	Formulation 1	Formulation 2	Formulation 3
Propofol	10 mg/mL	10 mg/mL	10 mg/mL
CAPTISOL® cyclodextrin	220 mg/mL	220 mg/mL	220 mg/mL
EDTA	-	0.05 mg/mL	-
Sodium Metabisulfite	-	-	0.25 mg/mL
pH	no adjustment	8.2	5.5
Sterile Water for Injection	to volume	to volume	to volume

EXAMPLE 2

The following example describes an exemplary method for the preparation of a preserved formulation according to the invention as a solid for reconstitution.

Ingredient	Amount
Propofol	20 mg/mL
CAPTISOL® cyclodextrin	432 mg/mL
Water	to volume

CAPTISOL® cyclodextrin was dissolved in water to form a solution containing about 0.2 Molar (approximately 432 mg/mL) of CAPTISOL® cyclodextrin. Propofol was then added to the SAE-CD containing solution with stirring until a concentration of about 20 mg/mL propofol was reached. The solution was lyophilized to generate a solid formulation. Prior to use as a solution, sufficient sterile water for injection is added to the solid formulation to generate a final solution containing propofol 10 mg/mL.

EXAMPLE 3

The growth retarding capability of three formulations of the invention, formulations 1, 2, and 3 from example 1, were compared to two marketed formulations of the active agent that do not contain a cyclodextrin of the invention. The two marketed formulations, Diprivan Injectable Emulsion 1% and Propofol Injectable Emulsion 1% each contain 10 mg/mL propofol, 100 mg/mL soybean oil, 22.5 mg/mL glycerol, and 12 mg/mL egg lecithin. In addition, Diprivan Injectable Emulsion 1% contains 0.05 mg/mL disodium edetate (EDTA) at a pH of 7.0 to 8.5, and Propofol Injectable Emulsion 1% contains 0.25 mg/mL sodium metabisulfite at a pH of 4.5 to 6.4. The antimicrobial

preserving capability of the formulations was evaluated in duplicate employing a liquid to liquid matrix against seven test organisms. at three exposure intervals, and at two exposure temperatures, then quantitated using membrane filtration. Approximately 50-200 colony formation units (CFU) per mL of five standard organisms recommended by United States Pharmacopoeia (USP) for preservative efficacy tests were inoculated in each formulation. These five organisms are identified as *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231). In addition to these organisms, *Staphylococcus epidermis* (ATCC 12228) and methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 700698) were also tested.

After the samples were incubated at 20-25°C or 30-35°C, they were inoculated with the test organisms to yield approximately 50-200 colony forming units (CFU)/mL. The viable count of the test organism was determined immediately following inoculation, and then after 24 and 48 hours of exposure. An aliquot of the test sample-cell suspension was suspended with peptone Tween® solution, filtered using a 0.45 μm filter, washed with a peptone Tween® solution and the membrane was transferred to a neutralized agar plate. The plates were incubated at 35-39°C for 24-72 hours for bacteria, at 20-25°C for 48-72 hours for the yeast, and at 20-25°C for 4-10 days for the mold. This study was verified using a neutralization procedure to ensure that the recovery medium is capable of neutralizing any residual preservative when the test aliquots were drawn and filtered.

The following Tables 1-14 compare the antimicrobial effectiveness of Captisol® cyclodextrin solutions of propofol with that of DIPRIVAN® Injectable Emulsion 1% and Propofol Injectable Emulsion 1% solutions. These results indicate that formulations of propofol containing Captisol® cyclodextrin possess microbial growth retarding or preservative properties and are capable of decreasing the content of viable microorganisms for at least 48 hours after adventitious, extrinsic contamination with bacteria, yeast and mold. The emulsion formulations supported the growth of each of the bacteria, allowing increased numbers of viable microorganisms to be present after the incubation period.

TABLE 1

Comparison of microbial growth retarding activity of various formulations against *S. aureus* (ATCC 6538) incubation at 20-25°C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.59 ± 0.03	ND	ND	2.59	2.59
Captisol® Formulation 2	2.63 ± 0.02	ND	ND	2.63	2.63
Captisol® Formulation 3	2.65 ± 0.02	ND	ND	2.65	2.65
Diprivan Injectable Emulsion 1%	2.69 ± 0.04	4.31 ± 0.01	6.78 ± 0.11	NA	NA
Propofol Injectable Emulsion 1%	2.68 ± 0.06	4.35 ± 0.33	5.60 ± 0.08	NA	NA

NA: Not applicable, ND: No viable organisms detected in 1 mL aliquot, SD: standard deviation.

TABLE 2

Comparison of microbial growth retarding activity of various formulations against *S. aureus* (ATCC 6538) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.63 ± 0.01	ND	ND	2.63	2.63
Captisol® Formulation 2	2.65 ± 0.03	ND	ND	2.65	2.65
Captisol® Formulation 3	2.65 ± 0.01	ND	ND	2.65	2.65
Diprivan Injectable Emulsion 1%	2.54 ± 0.01	5.23 ± 0.44	6.56 ± 0.09	NA	NA
Propofol Injectable Emulsion 1%	2.63 ± 0.06	5.79 ± 0.30	6.89 ± 0.01	NA	NA

TABLE 3

Comparison of microbial growth retarding activity of various formulations against *E. coli* (ATCC 8739) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.47 ± 0.04	0.96 ± 1.35	ND	1.51	2.47
Captisol® Formulation 2	2.58 ± 0.04	0.30 ± 0.42	ND	2.28	2.58
Captisol® Formulation 3	2.56 ± 0.07	0.15 ± 0.21	0.52 ± 0.74	2.41	2.04
Diprivan Injectable Emulsion 1%	2.53 ± 0.08	5.96 ± 0.02	7.98 ± 0.03	NA	NA
Propofol Injectable Emulsion 1%	2.58 ± 0.01	6.40 ± 0.04	7.81 ± 0.33	NA	NA

TABLE 4

Comparison of microbial growth retarding activity of various formulations against *E. coli* (ATCC 8739) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.50 ± 0.01	ND	ND	2.50	2.50
Captisol® Formulation 2	2.46 ± 0.04	ND	ND	2.46	2.46
Captisol® Formulation 3	2.57 ± 0.05	ND	ND	2.57	2.57
Diprivan Injectable Emulsion 1%	2.67 ± 0.01	6.90 ± 0.84	7.92 ± 0.08	NA	NA
Propofol Injectable Emulsion 1%	2.71 ± 0.04	7.76 ± 0.28	7.68 ± 0.67	NA	NA

TABLE 5

Comparison of microbial growth retarding activity of various formulations against *C. albicans* (ATCC 10231) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.45 ± 0.01	ND	ND	2.45	2.45
Captisol® Formulation 2	2.41 ± 0.03	ND	ND	2.41	2.41
Captisol® Formulation 3	2.44 ± 0.08	ND	ND	2.44	2.44
Diprivan Injectable Emulsion 1%	2.39 ± 0.04	2.46 ± 0.07	2.50 ± 0.18	NA	NA
Propofol Injectable Emulsion 1%	2.43 ± 0.01	3.14 ± 0.86	3.55 ± 1.27	NA	NA

TABLE 6

Comparison of microbial growth retarding activity of various formulations against *C. albicans* (ATCC 10231) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.45 ± 0.06	ND	ND	2.45	2.45
Captisol® Formulation 2	2.41 ± 0.04	ND	ND	2.41	2.41
Captisol® Formulation 3	2.43 ± 0.00	ND	ND	2.43	2.43
Diprivan Injectable Emulsion 1%	2.50 ± 0.05	2.58 ± 0.23	2.81 ± 0.57	NA	NA
Propofol Injectable Emulsion 1%	2.50 ± 0.03	3.17 ± 0.76	4.69 ± 0.05	NA	NA

TABLE 7

Comparison of microbial growth retarding activity of various formulations against *S. epidermis* (ATCC 12228) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.37 ± 0.04	ND	ND	2.37	2.37
Captisol® Formulation 2	2.38 ± 0.06	ND	ND	2.38	2.38
Captisol® Formulation 3	2.38 ± 0.02	ND	ND	2.38	2.38
Diprivan Injectable Emulsion 1%	2.43 ± 0.02	2.47 ± 0.08	2.43 ± 0.14	NA	NA
Propofol Injectable Emulsion 1%	2.40 ± 0.00	2.59 ± 0.11	2.68 ± 0.11	NA	NA

TABLE 8

Comparison of microbial growth retarding activity of various formulations against *S. epidermis* (ATCC 12228) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.37 ± 0.02	ND	ND	2.37	2.37
Captisol® Formulation 2	2.35 ± 0.06	ND	ND	2.35	2.35
Captisol® Formulation 3	2.39 ± 0.04	ND	ND	2.39	2.39
Diprivan Injectable Emulsion 1%	2.40 ± 0.01	2.63 ± 0.00	2.75 ± 0.04	NA	NA
Propofol Injectable Emulsion 1%	2.34 ± 0.05	5.44 ± 0.33	7.52 ± 0.34	NA	NA

TABLE 9

Comparison of microbial growth retarding activity of various formulations against *S. aureus* (MSRA) (ATCC 700698) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.36 ± 0.04	ND	ND	2.36	2.36
Captisol® Formulation 2	2.38 ± 0.07	ND	ND	2.38	2.38
Captisol® Formulation 3	2.32 ± 0.02	ND	ND	2.32	2.32
Diprivan Injectable Emulsion 1%	2.57 ± 0.08	3.52 ± 0.06	5.73 ± 1.12	NA	NA
Propofol Injectable Emulsion 1%	2.43 ± 0.03	3.48 ± 0.72	5.24 ± 0.28	NA	NA

TABLE 10

Comparison of microbial growth retarding activity of various formulations against *S. aureus* (MSRA) (ATCC 700698) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.40 ± 0.02	ND	ND	2.40	2.40
Captisol® Formulation 2	2.33 ± 0.01	ND	ND	2.33	2.33
Captisol® Formulation 3	2.39 ± 0.01	ND	ND	2.39	2.39
Diprivan Injectable Emulsion 1%	2.47 ± 0.06	4.23 ± 0.21	6.06 ± 0.84	NA	NA
Propofol Injectable Emulsion 1%	2.41 ± 0.00	5.68 ± 0.28	6.31 ± 0.22	NA	NA

TABLE 11

Comparison of microbial growth retarding activity of various formulations against *P. aeruginosa* (ATCC 9027) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.21 ± 0.03	0.89 ± 1.25	0.93 ± 1.32	1.32	1.28
Captisol® Formulation 2	2.10 ± 0.00	0.88 ± 1.24	ND	1.22	2.10
Captisol® Formulation 3	2.21 ± 0.03	ND	ND	2.21	2.21
Diprivan Injectable Emulsion 1%	2.42 ± 0.01	5.14 ± 0.08	7.97 ± 0.11	NA	NA
Propofol Injectable Emulsion 1%	2.32 ± 0.06	5.45 ± 0.02	7.86 ± 0.07	NA	NA

TABLE 12

Comparison of microbial growth retarding activity of various formulations against *P. aeruginosa* (ATCC 9027) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	2.29 ± 0.02	ND	ND	2.29	2.29
Captisol® Formulation 2	2.07 ± 0.03	ND	ND	2.07	2.07
Captisol® Formulation 3	2.09 ± 0.03	1.80 ± 0.30	1.97 ± 0.51	0.29	0.12
Diprivan Injectable Emulsion 1%	2.32 ± 0.06	5.08 ± 0.00	8.02 ± 0.08	NA	NA
Propofol Injectable Emulsion 1%	2.21 ± 0.03	8.27 ± 0.01	8.38 ± 0.05	NA	NA

TABLE 13

Comparison of microbial growth retarding activity of various formulations against *A. niger* (ATCC 16404) incubation at 20-25 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	0.95 ± 0.00	0.15 ± 0.21	ND	0.8	0.95
Captisol® Formulation 2	0.93 ± 0.04	0.24 ± 0.34	0.24 ± 0.34	0.69	0.69
Captisol® Formulation 3	0.93 ± 0.11	0.30 ± 0.42	0.54 ± 0.34	0.63	0.39
Diprivan Injectable Emulsion 1%	1.08 ± 0.05	0.72 ± 0.33	0.54 ± 0.08	0.36	0.54
Propofol Injectable Emulsion 1%	0.59 ± 0.39	ND	ND	0.59	0.59

TABLE 14

Comparison of microbial growth retarding activity of various formulations against *A. niger* (ATCC 16404) incubation at 30-35 °C.

Formulation	Viable count of Survivors log_{10} CFU/mL ± SD			Decrease in survivors log_{10} CFU/mL	
	0 h	24h	48h	24h	48h
Captisol® Formulation 1	0.63 ± 0.46	0.59 ± 0.16	0.39 ± 0.13	0.04	0.24
Captisol® Formulation 2	0.84 ± 0.08	0.63 ± 0.21	0.43 ± 0.60	0.21	0.41
Captisol® Formulation 3	0.80 ± 0.14	0.72 ± 0.33	0.24 ± 0.34	0.08	0.56
Diprivan Injectable Emulsion 1%	0.73 ± 0.18	0.91 ± 0.18	0.30 ± 0.42	NA	0.43
Propofol Injectable Emulsion 1%	0.78 ± 0.11	ND	ND	0.78	0.78

EXAMPLE 4

Water activity was measured by placing a sample solution in a small, sealed container and determining the equilibrium humidity and temperature in the container. Instruments such as the HygroLab 3 from Rotronic Instrument Corp., Huntington, NY were used to measure the water activity. The humidity is determined using a thin film capacitive sensor in the headspace of the container. The temperature is determined using a Pt RTD 100 sensor. From these measurements the activity of water (a_w) is calculated by the instrument. The instrument has an accuracy of about ±0.015 a_w and a reproducibility

of about ±0.005 a_w. Carefully prepared salt-containing stock solutions of known concentration and water activity were used to calibrate the instrument prior to use.

EXAMPLE 5

The following general method, which is similar to the method of Examples 3 or 6, is used to evaluate the preservation of aqueous formulations comprising water, a derivatized cyclodextrin, optionally an active agent, and a water activity-reducing agent.

Water activity approximation

A composition is prepared by mixing known amounts of water, derivatized cyclodextrin and water activity-reducing agent optionally in the presence of heat. The water activity of the aqueous composition is measured according to Example 4. Depending upon the value of water activity, the composition is then evaluated according to the procedures in the appropriate pharmacopoeia or Examples 3 or 6 to determine a performance rating in preserving activity against a target microbe. For example, if the water activity approximates or is less than 0.97 ± 0.025, then the composition is optionally evaluated according to Examples 3 or 6to determine its suitability for use according to the invention. Depending upon the particular microbe being tested for, a different water activity value may be used as the initial screening value. For example, a water activity value of less than about 0.96±0.025 may be used to screen formulations containing water, a derivatized cyclodextrin, a water activity-reducing agent and *E. coli*. Also, a water activity value of less than about 0.95±0.025 may be used to screen formulations containing water and a derivatized cyclodextrin for use in a preserved formulation. In addition, the target water activity value may vary according to the derivatized cyclodextrin being used in the test.

EXAMPLE 6

Formulation with at least a substantial portion of drug bound to SAE-CD:

Exemplary formulations according to the invention were made according to the following general procedure. A derivatized cyclodextrin was dissolved in water to form a solution containing about 400 mg/mL of the cyclodextrin. An active agent known to complex with the cyclodextrin was optionally added to the cyclodextrin containing solution until a concentration of about 20 mg/mL active agent was reached. The solution

was sterilized by filtration with a 0.2 μm filter. The following formulations were prepared according to this procedure.

Ingredient	Amount Formulation 4	Formulation 5	Formulation 6	Formulation 7
Ibuprofen	-	20 mg/mL	-	20 mg/mL
CAPTISOL® cyclodextrin	400 mg/mL	400 mg/mL	-	-
2-Hydroxypropyl-β-cyclodextrin (DS=4.3)	-	-	400 mg/mL	400 mg/mL
Sterile Water for Injection	to volume	to volume	to volume	to volume

The antimicrobial preserving capability of formulations 4-7 was evaluated by testing each formulation using the procedure of USP XXV <51> test for preservative effectiveness except that the time points analyzed were 0, 24, and 72 hours post inoculation. In this test, aliquots of each formulation were inoculated at ~1 x 10^6 colony forming units (CFU) of the following organisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) and the non-USP microbe, *Salmonella choleraesuis* (ATTC 13311). The log reduction in microbial content for each formulation is given for each time point in the table below. As shown in the table, the binding of the derivatized cyclodextrin of the invention to an active ingredient does not adversely affect the antimicrobial properties of the cyclodextrin solution.

Log Reductions in Microbial Content of 40% Cyclodextrin Solutions Neat and With 20 mg/mL Ibuprofen

	Determined Log Reductions in Microbial Content					
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger	S. choleraesuis
0 Hour						
Formulation 4	-0.1	0.3	0.2	0.1	0.1	0.2
Formulation 5	0.1	0.3	0.2	0.3	0.2	0.2
Formulation 6	0.1	0.1	0.1	0.3	0.2	0.2
Formulation 7	0.0	0.1	0.1	0.3	0.2	0.2
24 Hour						
Formulation 4	1.1	1.0	1.2	0.1	0.2	0.5
Formulation 5	1.0	0.2	0.3	0.2	0.1	0.2

	Determined Log Reductions in Microbial Content					
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger	S. choleraesuis
Formulation 6	1.1	-0.7	-0.5	0.2	0.1	0.1
Formulation 7	1.0	0.1	-0.1	0.1	0.1	0.1
72 Hour						
Formulation 4	1.3	2.2	1.5	0.2	0.1	0.7
Formulation 5	1.7	2.9	1.6	0.5	0.2	1.1
Formulation 6	1.4	-1.2	-0.6	0.2	0.3	0.2
Formulation 7	1.4	0.0	-0.8	0.2	0.2	-0.1

EXAMPLE 7

Formulation with drug mostly or completely unbound to SAE-CD.

Exemplary formulations according to the invention were made according to the following general procedure. A derivatized cyclodextrin was dissolved in water to form a solution containing about 400 mg/mL of the cyclodextrin. An active agent known to complex poorly or not at all with the cyclodextrin was optionally added to the cyclodextrin containing solution until a concentration of about 10 mg/mL active agent was reached. The solution was sterilized by filtration with a 0.2 μm filter. The following formulations were prepared according to this procedure.

Ingredient	Amount			
	Formulation 8	Formulation 9	Formulation 10	Formulation 11
Cyclopentolate HCl	-	10 mg/mL	-	10 mg/mL
CAPTISOL® cyclodextrin	400 mg/mL	400 mg/mL	-	-
2-Hydroxypropyl-β-cyclodextrin (DS=4.3)	-	-	400 mg/mL	400 mg/mL
Sterile Water for Injection	to volume	to volume	to volume	to volume

The antimicrobial preserving capability of formulations 8-11 was evaluated by testing each formulation using the procedure of USP XXV <51> test for preservative effectiveness except that the time points analyzed were 0, 24, and 72 hours post inoculation. In this test, aliquots of each formulation were inoculated at ~1 x 10^6 colony forming units (CFU) of the following organisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Aspergillus niger*

(ATCC 16404) *Candida albicans* (ATCC 10231) and the non-USP microbe, *Salmonella choleraesuis* (ATTC 13311). The log reduction in microbial content for each formulations is given for each time point in the table below. As shown in the table, the presence of an active agent that does not bind to the derivatized cyclodextrin of the invention does not adversely affect the antimicrobial properties of the cyclodextrin solution.

Log Reductions in Microbial Content of 40% Cyclodextrin Solutions Neat and With 10 mg/mL Cyclopentolate HCl

	Determined Log Reductions in Microbial Content					
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger	S. choleraesuis
0 Hour						
Formulation 8	-0.1	0.3	0.2	0.1	0.1	0.2
Formulation 9	0.1	0.2	0.2	0.2	0.1	0.2
Formulation 10	0.1	0.1	0.1	0.3	0.2	0.2
Formulation 11	0.1	0.1	0.0	0.3	0.3	0.1
24 Hour						
Formulation 8	1.1	1.0	1.2	0.1	0.2	0.5
Formulation 9	1.0	0.9	1.2	0.1	0.2	0.5
Formulation 10	1.1	-0.7	-0.5	0.2	0.1	0.1
Formulation 11	1.2	1.4	1.0	0.3	0.3	0.6
72 Hour						
Formulation 8	1.3	2.2	1.5	0.2	0.1	0.7
Formulation 9	1.5	2.6	1.7	0.3	0.2	0.9
Formulation 10	1.4	-1.2	-0.6	0.2	0.3	0.2
Formulation 11	1.5	0.4	1.0	0.2	0.1	0.4

The log values within the tables herein may vary according to the specific experimental conditions under which the corresponding assays were performed. For this reason, biostatic or biocidal activity is determined as compared to control samples and initial bioburden values.

EXAMPLE 8

Formulation with cooperative activity of SAE-CD and drug.

A 20% w/v neat Captisol solution and a 10 mg/mL propofol formulation containing 20% w/v Captisol plus 0-0.5% w/v citric acid were inoculated with 10^6 cfu/mL of the microorganisms listed below. The antimicrobial preserving capability of the formulations was evaluated by testing each formulation using the procedure of USP XXV <51> test for preservative effectiveness except that the time points analyzed were 0, 24,

and 72 hours post inoculation. The log reduction in microbial content for each formulations is given for each time point in the table below. As shown in the table, formulations containing both CAPTISOL cyclodextrin and the active agent propofol demonstrate antimicrobial preserving activity..

Time Interval	Captisol % w/v	Propofol % w/v	Citric Acid % w/v	Log Reductions S. aureus gram + bacteria	P. aeruginosa gram - bacteria	E. coli gram - bacteria	C. albicans yeast	A. niger mold	S. choleraesuis gram - bacteria
0 Hour									
	20	0	0	0	0.2	0.1	0.3	0.2	0.1
	20	1	0	1.6	1.8	2.3	2.8	0.2	>3.7
	20	1	0.1	1.5	3.8	2.5	3.2	0.4	>3.7
	20	1	0.3	1.5	>3.6	2.2	2.8	0.3	>3.7
	20	1	0.5	2.3	>3.7	2.5	>3.5	0.4	>3.7
24 Hour									
	20	0	0	0.1	0.2	-0.3	0.3	0.2	0
	20	1	0	>3.7	2.9	2.3	>4.0	0.4	>3.7
	20	1	0.1	>3.7	>3.6	2.5	>4.0	0.3	>3.7
	20	1	0.3	>3.7	>3.7	2.9	>4.0	0.3	>3.7
	20	1	0.5	>3.7	>3.7	2.7	>3.9	0.3	>3.7
72 Hour									
	20	0	0	0.2	0.2	-0.8	0.4	0.1	-0.4
	20	1	0	>3.7	>3.7	1.2	>4.0	0.3	>3.7
	20	1	0.1	>3.7	1.9	>1.8	>4.0	0.4	>3.7
	20	1	0.3	>3.7	>3.7	2.8	>4.0	0.2	>3.7
	20	1	0.5	>3.7	>3.3	3.3	>3.9	0.4	>3.7

EXAMPLE 9

Exemplary formulations according to the invention were made according to the following general procedure. Captisol® cyclodextrin was dissolved in water to form solutions containing about 48 to 500 mg/mL of the cyclodextrin. The solutions were sterilized by filtration with a 0.2 μm filter.

The antimicrobial preserving activity of the formulations was evaluated by testing each formulation using the procedure of USP XXV <51> test for preservative effectiveness except that the time points analyzed were 0, 24, 48, and 72 hours post inoculation. In this test, aliquots of each formulation were inoculated at ~1 x 10^6 colony forming units (CFU) of the following organisms: *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Aspergillus niger* (ATCC 16404) *Candida albicans* (ATCC 10231) and the non-USP microbe, *Salmonella choleraesuis* (ATTC 13311). The log reduction in microbial content determined for each formulation is given for each time point in the table below.

% w/v Captisol Concentration	Determined Log Reductions in Microbial Content					
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger	S. choleraesuis
0 Hour						
4.8	-0.2	-0.6	-0.4	-0.1	-0.1	-0.1
9.5	-0.6	-0.6	-0.7	-0.2	0.0	-0.4
20.0	0.0	0.2	0.1	0.3	0.2	0.1
25.6	-0.1	-0.1	-0.1	0.0	0.0	-0.1
30.0	0.0	0.4	0.2	0.3	0.3	0.2
34.7	0.0	0.3	0.1	0.1	0.1	0.1
38.6	-0.1	0.2	-0.1	-0.1	0.0	-0.1
49.3	-0.1	0.2	-0.1	0.0	0.1	-0.1
24 Hour						
4.8	0.1	-0.5	-0.7	0.5	0.4	-0.1
9.5	0.1	-0.6	-0.8	0.1	0.3	-0.1
20.0	0.1	0.2	-0.3	0.3	0.2	0.0
25.6	0.2	0.4	-0.4	0.0	0.3	-0.1
30.0	0.9	0.7	0.2	0.1	0.2	0.1
34.7	1.4	1.0	0.9	0.0	0.1	0.2
38.6	3.1	1.2	1.5	0.0	0.4	0.3
49.3	>3.8	2.1	1.5	0.1	0.3	0.7
48 Hour						
4.8	0.1	-0.7	-0.8	0.6	0.4	-0.3
9.5	0.2	-0.9	-0.8	0.1	0.3	-0.2
20.0	ND	ND	ND	ND	ND	ND
25.6	0.5	0.4	-0.6	0.0	0.4	0.0
30.0	1.3	0.9	0.5	0.1	0.2	0.2
34.7	1.6	1.2	1.1	0.1	0.1	0.2
38.6	3.4	2.0	1.7	0.1	0.3	0.6
49.3	>3.7	3.0	2.1	0.3	0.3	1.0
72 Hour						
4.8	0.3	-1.0	-1.0	0.6	0.4	-0.5
9.5	0.3	-1.0	-1.1	0.1	-0.1	-0.6
20.0	0.2	0.2	-0.8	0.4	0.1	-0.4
25.6	0.6	-0.8	-0.9	0.2	0.3	0.0
30.0	1.5	0.7	0.5	0.2	0.1	0.1
34.7	1.6	1.4	1.1	0.2	0.0	0.3
38.6	>3.7	>3.4	1.7	0.3	0.3	0.3
49.3	>4.0	3.7	2.4	0.5	0.3	1.2

≤ -0.5 ;pg reduction = microbial growth, -0.5 to 0.5 log reduction = microbiostatic, ≥ 0.5 log reduction = microbicidal.

EXAMPLE 10

The following %w/v Captisol solutions were prepared and the water activity of each determined prior to being sent for antimicrobial testing. The measured water activity

of the %w/v Captisol solutions agree with the water activity values measured from the %w/w Captisol solutions above. The table below lists the measured water activity and the location of the corresponding antimicrobial testing results

Captisol Conc. %w/v	Measured a_w	Antimicrobial Testing Results
20	0.974	See Example #8 or 9
30	0.962	See Example #9
40	0.952	See Example#6 (or #7)

The disclosures of the references cited herein are hereby incorporated in their entirety.

The above is a detailed description of particular embodiments of the invention. It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims. All of the embodiments disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure.

CLAIMS

1. A method of preserving a liquid formulation against microbial proliferation comprising the step of:

including a sulfoalkyl ether cyclodextrin in the formulation in an amount sufficient to preserve the formulation by at least reducing the rate of increase of microbial bioburden that may be present in the formulation during storage for at least a predetermined period of time, wherein the formulation optionally comprises at least one active agent.

2. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 25% wt/vol based upon the total volume of the formulation.

3. The method of claim 2, wherein the cyclodextrin provides growth retarding, biostatic and/or biocidal properties to the formulation.

4. The method of claim 3, wherein the cyclodextrin at least reduces the rate of bioburden increase in the formulation as compared to a control formulation excluding the cyclodextrin.

5. The method of claim 3, wherein the cyclodextrin at least maintains a substantially constant bioburden in the formulation during storage of the formulation.

6. The method of claim 3, wherein the cyclodextrin at least reduces the bioburden of the formulation during storage of the formulation.

7. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 25 % wt./vol of the formulation and possesses biocidal activity.

8. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 4.8% wt/vol of the formulation and possesses biostatic activity.

9. The method of claim 1 further comprising the step of including one or more water activity-reducing agents in the formulation.

10. The method of claim 2, wherein the active agent is present and the cyclodextrin is present in molar excess over the active agent.

11. The method of claim 10, wherein a major portion of the active agent is not complexed with the cyclodextrin.

12. The method of claim 10, wherein a major portion of the active agent is complexed with the cyclodextrin by formation of an inclusion complex therewith and/or formation of a salt therewith.

13. The method of claim 10, wherein the cyclodextrin increases the concentration of dissolved active agent in the formulation.

14. The method of claim 1 further comprising the step of sterile filtering the formulation through a filtration medium having a pore size of 0.1 microns or larger.

15. The method of claim 1 further comprising the step of isolating a reconstitutable solid from the liquid formulation, wherein the solid comprises an active agent, cyclodextrin and optionally a pharmaceutically acceptable excipient.

16. The method of claim 1 further comprising the step of purging the formulation with an inert pharmaceutically acceptable gas such that at least a substantial portion of the oxygen dissolved in the formulation is removed.

17. The method of claim 1, wherein the formulation has a water activity of less than about 0.97±0.025.

18. The method of claim 1, wherein the formulation has a water activity of less than about 0.95±0.025.

19. The method of claim 1, wherein the formulation has a water activity of less than about 0.90±0.025.

20. The method of claim 1, wherein the formulation has a water activity of less than about 0.85±0.025.

21. The method of claim 1, wherein the formulation has a water activity of less than about 0.80±0.025.

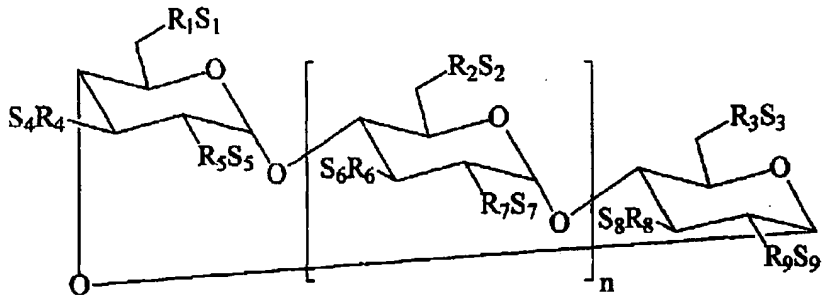
22. The method of claim 1 further comprising a conventional preservative.

23. The method of claim 1 wherein the cyclodextrin is present in an amount of at least about 40%±2.5% wt/vol based upon the total volume of the formulation.

24. The method of claim 23, wherein the cyclodextrin preserves the formulation at least against proliferation of one or more of *P. aeruginosa, E. coli, S. aureus, C. albicans,* and *A. niger*.

25. The method of claim 9, wherein the one or more water activity-reducing agents is selected from the group consisting of poly(vinyl pyrrolidone) and poly(ethylene glycol).

26. The method of claim 1-24 or 25 wherein the cyclodextrin is a compound of the Formula 1:



Formula 1

wherein:

n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of $R_1 - R_9$ is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, a -O-$(CH_2)_mSO_3^-$ group wherein m is 2 to 6, $-OCH_2CH_2CH_2SO_3^-$, or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

27. The method of claim 26, wherein the formulation further comprises at least one of antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavor or sweetener.

28. A method of unmasking a preservative property of an active agent not otherwise known to possess such property, the method comprising the step of exposing the active agent to sulfoalkyl ether cyclodextrin in an aqueous solution, optionally contaminated with a microbe, wherein the cyclodextrin is present in an amount sufficient to unmask

a preservative property of the drug, and the drug is present in an amount sufficient to provide a preservative effect against a microbe in the solution.

29. The method of claim 28, wherein the preservative property is selected from the group consisting of biocidal activity, growth retarding activity, biostatic activity, and a combination thereof.

30. The method of claim 29, wherein the active agent is a phenol-based active agent.

31. A method of preserving a liquid formulation comprising the step of:

including a sulfoalkyl ether cyclodextrin an optional active agent and a conventional preservative in the formulation wherein neither the cyclodextrin nor preservative is present in an amount sufficient to preserve the formulation independently, but wherein the cyclodextrin and preservative cooperate to provide a preservative effect in the formulation.

32. The method of claim 31, wherein the formulation is preserved by at least reducing the rate of increase of microbial bioburden that may be present in the formulation during storage for at least a predetermined period of time.

33. The method of claim 32, wherein the cyclodextrin is present in an amount less than about 30% wt/vol based upon the final volume of the formulation.

34. The method of claim 32, wherein the preservative and cyclodextrin together provide growth retarding, biostatic and/or biocidal properties to the formulation.

35. The method of claim 32, wherein the preservative and cyclodextrin together at least reduce the rate of bioburden increase in the formulation as compared to a control formulation excluding the cyclodextrin and/or preservative.

36. The method of claim 32, wherein the preservative and cyclodextrin together at least maintain a substantially constant bioburden in the formulation during storage of the formulation.

37. The method of claim 32, wherein the preservative and cyclodextrin together at least reduce the bioburden of the formulation during storage of the formulation.

38. The method of claim 32, wherein the active agent is present and the cyclodextrin is present in molar excess over the active agent.

39. The method of claim 38, wherein a major portion of the active agent is not complexed with the cyclodextrin.

40. The method of claim 38. wherein a major portion of the active agent is complexed with the cyclodextrin by formation of an inclusion complex therewith and/or formation of a salt therewith.

41. The method of claim 38, wherein the cyclodextrin increases the concentration of dissolved active agent in the formulation.

42. The method of claim 32, wherein the formulation has a water activity of less than about 0.97±0.025.

43. The method of claim 32, wherein the formulation has a water activity of less than about 0.95±0.025.

44. The method of claim 32, wherein the formulation has a water activity of less than about 0.90±0.025.

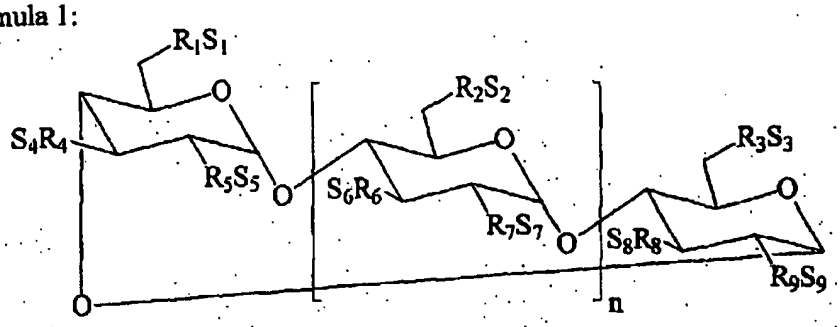
45. The method of claim 32, wherein the formulation has a water activity of less than about 0.85±0.025.

46. The method of claim 32, wherein the formulation has a water activity of less than about 0.80±0.025.

47. The method of claim 32, wherein the preservative and cyclodextrin together preserve the formulation at least against proliferation of one or more of *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*.

48. The method of claim 32 wherein the formulation comprises one or more water activity reducing agents, and the one or more water activity-reducing agents is selected from the group consisting of poly(vinyl pyrrolidone) and poly(ethylene glycol).

49. The method of claim 31-47 or 48. wherein the cyclodextrin is a compound of the Formula 1:



Formula 1

wherein:

n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of R_1 – R_9 is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, a -O-$(CH_2)_mSO_3^-$ group wherein m is 2 to 6, -$OCH_2CH_2CH_2SO_3^-$, or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

50. The method of claim 49, wherein the formulation further comprises at least one of antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavor or sweetener.

51. A method of preserving a liquid formulation comprising the step of:

including a cyclodextrin and/or cyclodextrin derivative, an optional active agent, and a water activity reducing agent in the formulation, wherein neither the cyclodextrin nor the water activity reducing agent is present in an amount sufficient to preserve the formulation independently, but wherein the cyclodextrin and water activity reducing agent cooperate to provide a preservative effect in the formulation.

52. The method of claim 51, wherein the cyclodextrin derivative is sulfoalkyl ether cyclodextrin.

53. The method of claim 51, wherein the cyclodextrin is alpha, beta or gamma cyclodextrin.

54. The method of claim 51, wherein the formulation is preserved by at least reducing the rate of increase of microbial bioburden that may be present in the formulation during storage for at least a predetermined period of time.

55. The method of claim 54, wherein the cyclodextrin and/or cyclodextrin derivative is present in an amount less than about 30% wt/vol based upon the final volume of the formulation.

56. The method of claim 54, wherein the preservative and the cyclodextrin and/or cyclodextrin derivative together provide growth retarding, biostatic and/or biocidal properties to the formulation.

57. The method of claim 54, wherein the preservative and the cyclodextrin and/or cyclodextrin derivative together at least reduce the rate of bioburden increase in the formulation as compared to a control formulation excluding the cyclodextrin, cyclodextrin derivative and/or preservative.

58. The method of claim 54, wherein the preservative and the cyclodextrin and/or cyclodextrin derivative together at least maintain a substantially constant bioburden in the formulation during storage of the formulation.

59. The method of claim 54, wherein the preservative and the cyclodextrin and/or cyclodextrin derivative together at least reduce the bioburden of the formulation during storage of the formulation.

60. The method of claim 54, wherein the active agent is present and the cyclodextrin and/or cyclodextrin derivative is present in molar excess over the active agent.

61. The method of claim 60, wherein a major portion of the active agent is not complexed with the cyclodextrin and/or cyclodextrin derivative.

62. The method of claim 60, wherein a major portion of the active agent is complexed with the cyclodextrin and/or cyclodextrin derivative by formation of an inclusion complex therewith and/or formation of a salt therewith.

63. The method of claim 60, wherein the cyclodextrin and/or cyclodextrin derivative increases the concentration of dissolved active agent in the formulation.

64. The method of claim 54, wherein the formulation has a water activity of less than about 0.97±0.025.

65. The method of claim 54, wherein the formulation has a water activity of less than about 0.95±0.025.

66. The method of claim 54, wherein the formulation has a water activity of less than about 0.90±0.025.

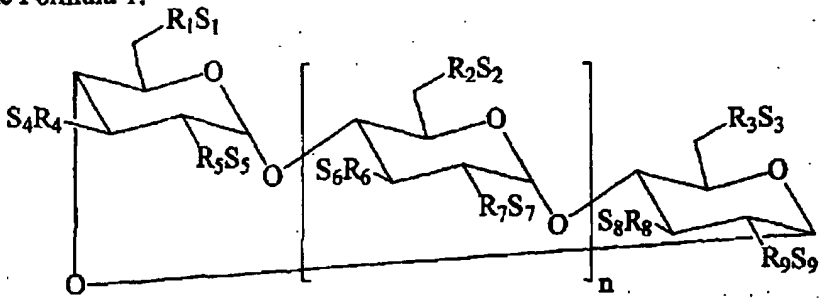
67. The method of claim 54, wherein the formulation has a water activity of less than about 0.85±0.025.

68. The method of claim 54, wherein the formulation has a water activity of less than about 0.80±0.025.

69. The method of claim 54, wherein the preservative and the cyclodextrin and/or cyclodextrin derivative together preserve the formulation at least against proliferation of one or more of *P. aeruginosa, E. coli, S. aureus, C. albicans*, and *A. niger*.

70. The method of claim 54, wherein the formulation comprises one or more water activity reducing agents, and the one or more water activity-reducing agents is selected from the group consisting of poly(vinyl pyrrolidone) and poly(ethylene glycol).

71. The method of claim 54-69 or 70, wherein the cyclodextrin derivative is a compound of the Formula 1:



Formula 1

wherein:

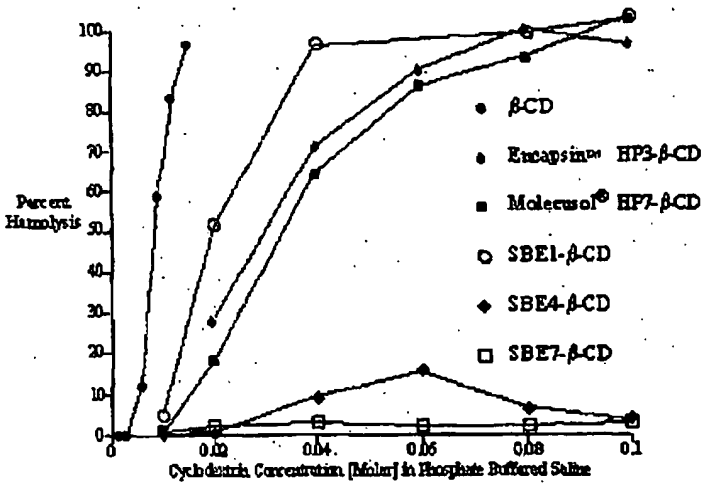
n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of R_1 – R_9 is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, a -O-$(CH_2)_mSO_3^-$ group wherein m is 2 to 6, -$OCH_2CH_2CH_2SO_3^-$, or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

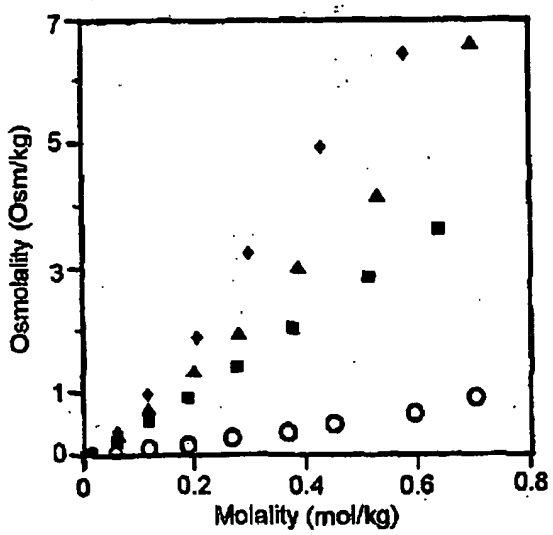
72. The method of claim 71, wherein the formulation further comprises at least one of antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavor or sweetener.

FIG. 1



PRIOR ART

FIG. 2



○-HPCD; ■-SBE4CD; ▲-SBE7CD; ♦-SBE9CD

FIG. 3

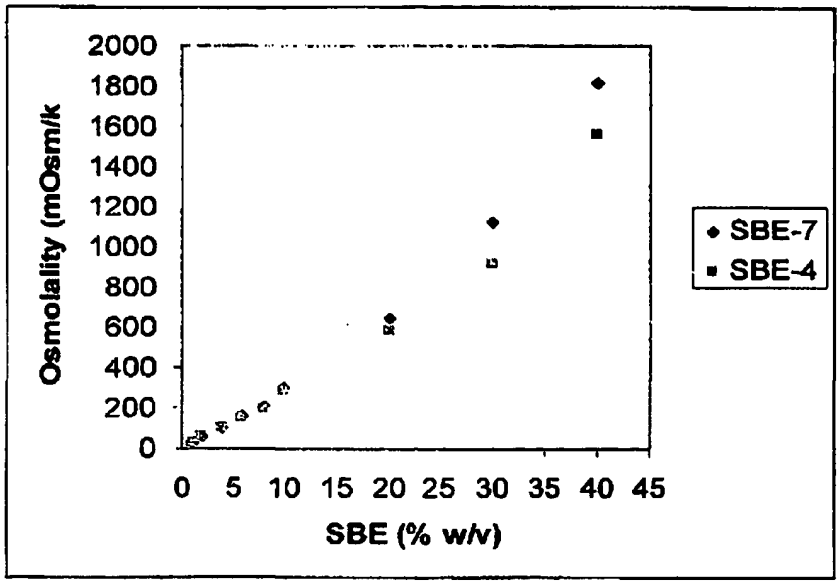

Osmolality (mOsm/k
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40 45
SBE (% w/v)
SBE-7
SBE-4

FIG. 4

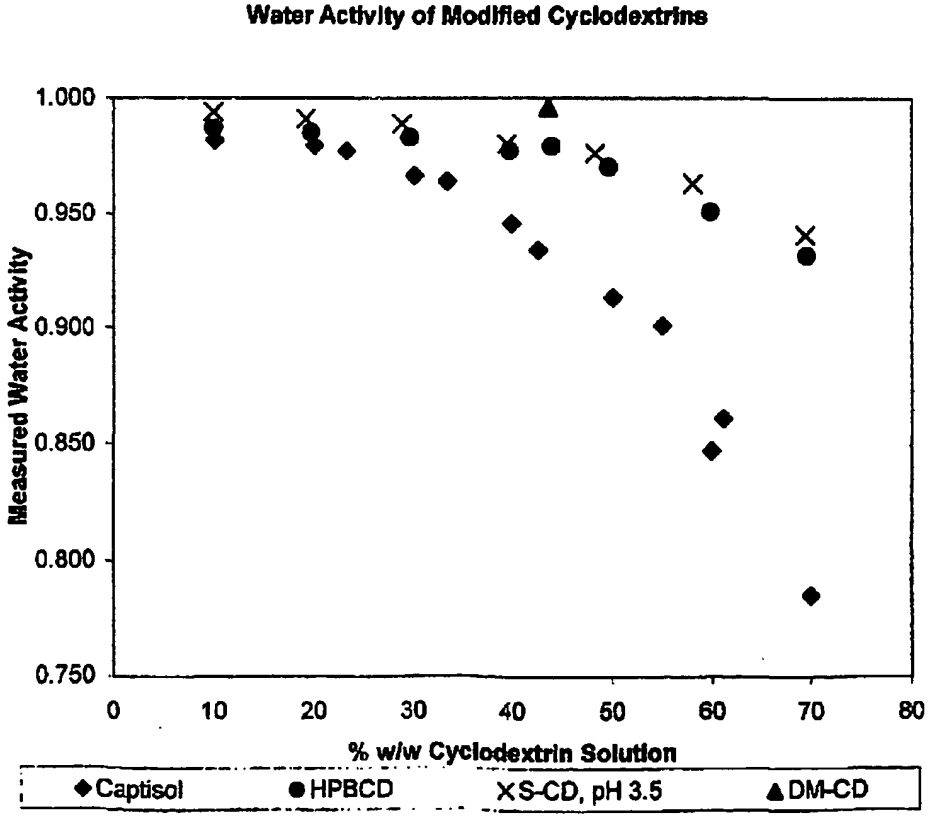

Water Activity of Modified Cyclodextrins
Measured Water Activity
1.000
0.950
0.900
0.850
0.800
0.750
0 10 20 30 40 50 60 70 80
% w/w Cyclodextrin Solution
Captisol
HPBCD
S-CD, pH 3.5
DM-CD

FIG. 5

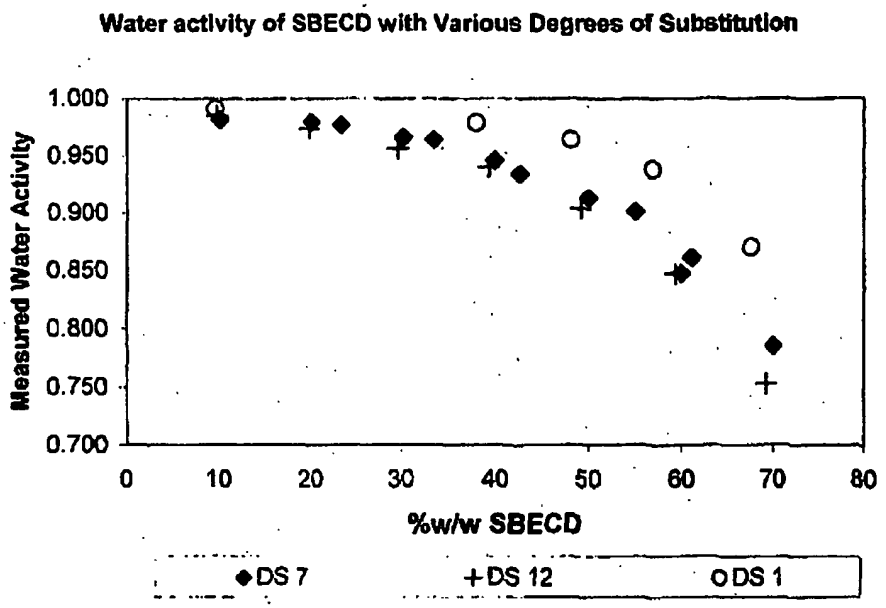

Water activity of SBECD with Various Degrees of Substitution
Measured Water Activity
1.000
0.950
0.900
0.850
0.800
0.750
0.700
0 10 20 30 40 50 60 70 80
%w/w SBECD
DS 7
DS 12
DS 1

FIG. 6

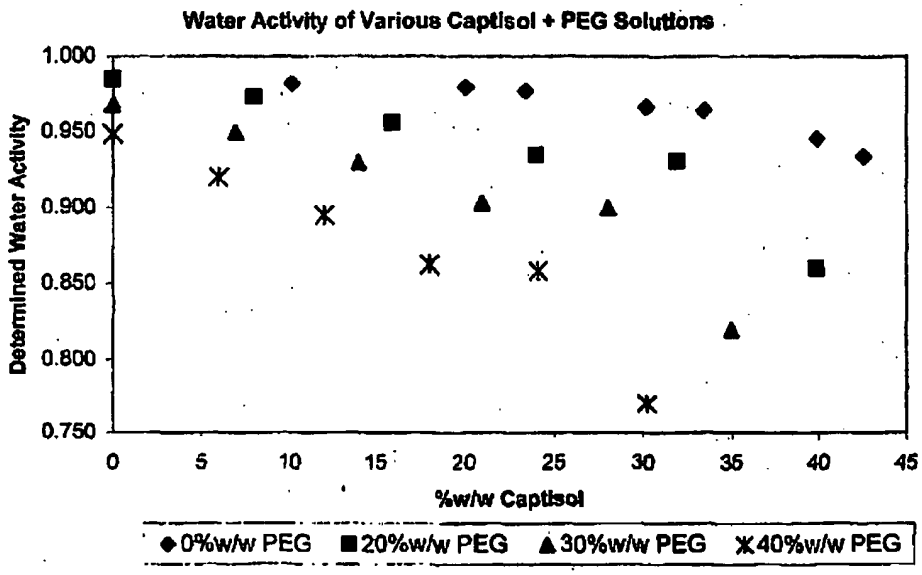

Water Activity of Various Captisol + PEG Solutions
Determined Water Activity
1.000
0.950
0.900
0.850
0.800
0.750
0 5 10 15 20 25 30 35 40 45
%w/w Captisol
0%w/w PEG
20%w/w PEG
30%w/w PEG
40%w/w PEG

FIG. 7

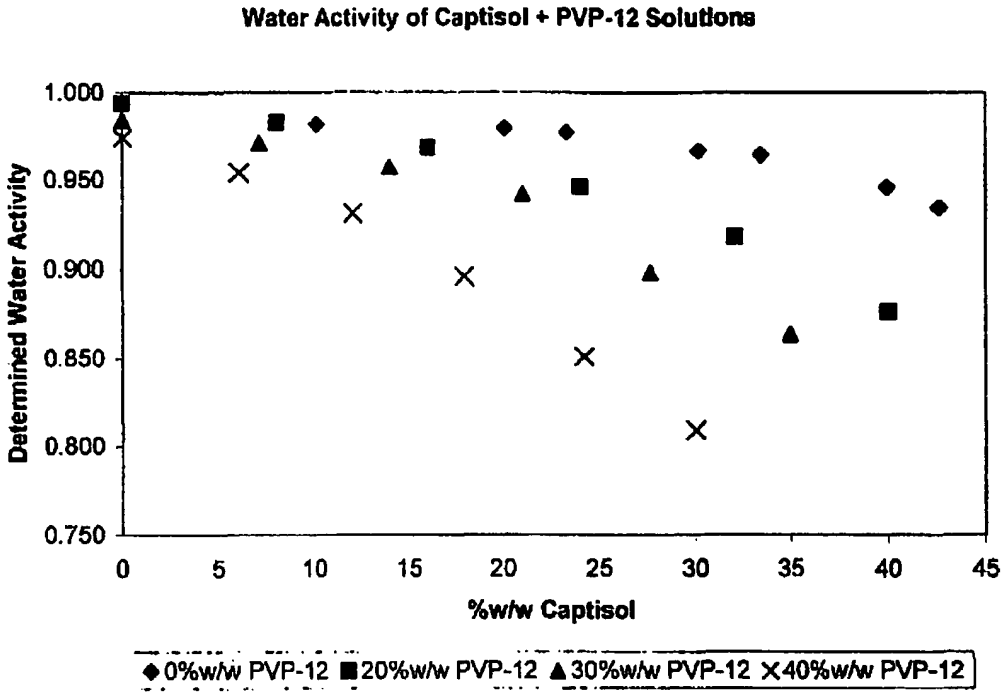
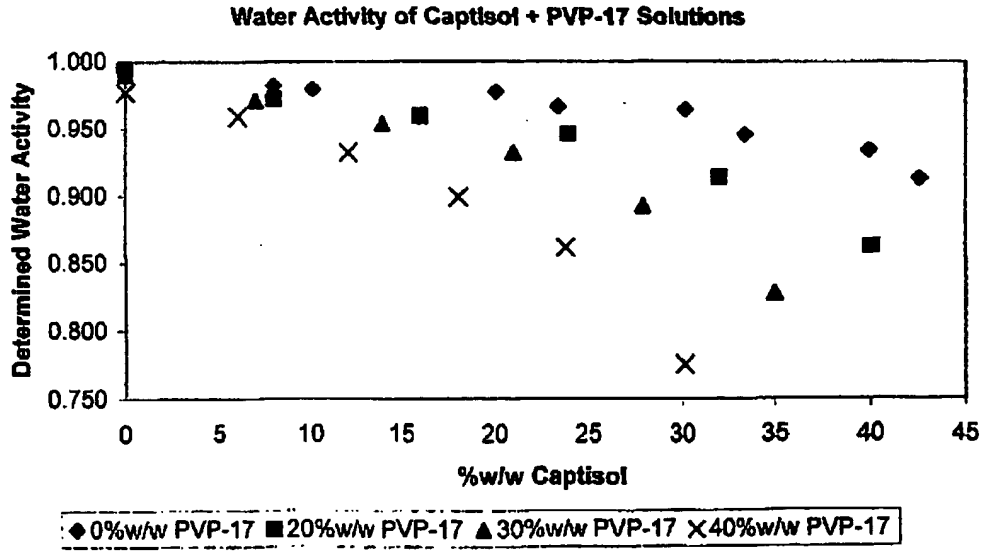

Water Activity of Captisol + PVP-12 Solutions
Determined Water Activity
1.000
0.950
0.900
0.850
0.800
0.750
0
5
10
15
20
25
30
35
40
45
%w/w Captisol
◆0%w/w PVP-12 ■20%w/w PVP-12 ▲30%w/w PVP-12 ×40%w/w PVP-12

FIG. 8


Water Activity of Captisol + PVP-17 Solutions
Determined Water Activity
1.000
0.950
0.900
0.850
0.800
0.750
0
5
10
15
20
25
30
35
40
45
%w/w Captisol
◆0%w/w PVP-17 ■20%w/w PVP-17 ▲30%w/w PVP-17 ×40%w/w PVP-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/08348

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/724; 47/40
US CL : 424/405; 514/58
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/405; 514/58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,232,304 B1 (KIM et al) 15 May 2001 (15.05.2001), cols 9-10.	1-27
X	US 5,506,216 A (SCHMIDT et al.) 09 April 1996 (09.04.1996), see entire reference.	51,53-70
Y		31-50, 52, 71, 72
Y	US 5,134,127 A (STELLA et al) 28 July 1992 (28.07.1992), see cols 3-4.	31-50, 52, 71, 72
A	US 5,985,310 A (CASTILLO et al) 16 November 1999 (16.11.1999), see entire reference.	1-72
A, P	US 6,358,935 B1 (BECK et al) 19 March 2002 (19.03.2002), see entire reference.	1-72
A, P	US 2002/0187960 A1 (SIKORSKI et al) 12 December 2002 (12.12.2002), see entire reference.	1-72

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 June 2003 (01.06.2003)	11 JUL 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230	Authorized officer Leigh C. Maier Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
EAST, CAPLUS
search terms: cyclodextrin, preservative, biocid?, biostat?, antimicrob?; water activity

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Published:
— *with international search report*
— *with amended claims and statement*

Date of publication of the amended claims and statement:
15 July 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/080079 A1

(54) Title: USE OF SULFOALKYL ETHER CYCLODEXTRIN AS A PRESERVATIVE

(57) Abstract: A method of preserving formulations is provided. The method includes the step of including a derivatized cyclodextrin in a formulation capable of sustaining microbial growth. One embodiment of the formulation employs a sulfoalkyl ether cyclodextrin as a preservative and optionally as a solubilizing and complexing agent. A suitable cyclodextrin is the CAPTISOL" brand cyclodextrin (sulfobutyl ether R-cyclodextrin). Whether or not the formulation includes a conventional preservative, the formulation will remain preserved for at least a minimum predetermined period. Specific embodiments of the invention include a carrier, a derivatized cyclodextrin and optionally one or more active agents, one or more water activity-reducing agents, and/or one or more complexation-enhancing agents. The derivatized cyclodextrin reduces the water activity of the formulation. A liquid formulation can be lyophilized or otherwise dried to yield a solid formulation that is optionally reconstitutable.

AMENDED CLAIMS
[received by the International Bureau on 05 September 2003 (05.09.2003);
original claims 1, 22, 31 and 51 amended;
remaining claims unchanged (6 pages)]

1. A method of preserving a liquid formulation, capable of sustaining microbial growth, against microbial proliferation comprising the step of:

including a sulfoalkyl ether cyclodextrin in the formulation in an amount sufficient to preserve the formulation by at least reducing the rate of increase of microbial bioburden that may be present in the formulation during storage for at least a predetermined period of time, wherein the formulation optionally comprises at least one active agent and wherein the formulation remains preserved in the absence of a conventional preservative.

2. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 25% wt/vol based upon the total volume of the formulation.

3. The method of claim 2, wherein the cyclodextrin provides growth retarding, biostatic and/or biocidal properties to the formulation.

4. The method of claim 3, wherein the cyclodextrin at least reduces the rate of bioburden increase in the formulation as compared to a control formulation excluding the cyclodextrin.

5. The method of claim 3, wherein the cyclodextrin at least maintains a substantially constant bioburden in the formulation during storage of the formulation.

6. The method of claim 3, wherein the cyclodextrin at least reduces the bioburden of the formulation during storage of the formulation.

7. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 25 % wt./vol of the formulation and possesses biocidal activity.

8. The method of claim 1, wherein the cyclodextrin is present in an amount of at least about 4.8% wt/vol of the formulation and possesses biostatic activity.

9. The method of claim 1 further comprising the step of including one or more water activity-reducing agents in the formulation.

10. The method of claim 2, wherein the active agent is present and the cyclodextrin is present in molar excess over the active agent.

11. The method of claim 10, wherein a major portion of the active agent is not complexed with the cyclodextrin.

12. The method of claim 10, wherein a major portion of the active agent is complexed with the cyclodextrin by formation of an inclusion complex therewith and/or formation of a salt therewith.

13. The method of claim 10, wherein the cyclodextrin increases the concentration of dissolved active agent in the formulation.

14. The method of claim 1 further comprising the step of sterile filtering the formulation through a filtration medium having a pore size of 0.1 microns or larger.

15. The method of claim 1 further comprising the step of isolating a reconstitutable solid from the liquid formulation, wherein the solid comprises an active agent, cyclodextrin and optionally a pharmaceutically acceptable excipient.

16. The method of claim 1 further comprising the step of purging the formulation with an inert pharmaceutically acceptable gas such that at least a substantial portion of the oxygen dissolved in the formulation is removed.

17. The method of claim 1, wherein the formulation has a water activity of less than about 0.97±0.025.

18. The method of claim 1, wherein the formulation has a water activity of less than about 0.95±0.025.

19. The method of claim 1, wherein the formulation has a water activity of less than about 0.90±0.025.

20. The method of claim 1, wherein the formulation has a water activity of less than about 0.85±0.025.

21. The method of claim 1, wherein the formulation has a water activity of less than about 0.80±0.025.

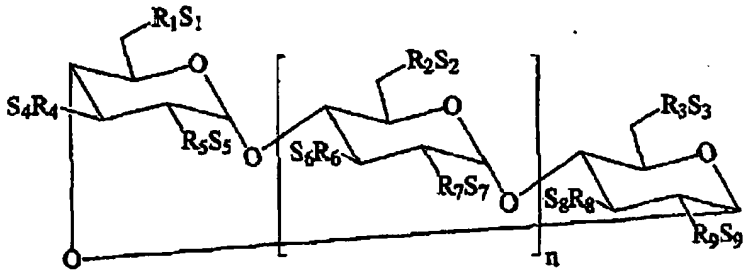
22. The method of claim 1 further comprising the step of including a conventional preservative in the liquid formulation.

23. The method of claim 1 wherein the cyclodextrin is present in an amount of at least about 40%±2.5% wt/vol based upon the total volume of the formulation.

24. The method of claim 23, wherein the cyclodextrin preserves the formulation at least against proliferation of one or more of *P. aeruginosa, E. coli, S. aureus, C. albicans,* and *A. niger*.

25. The method of claim 9, wherein the one or more water activity-reducing agents is selected from the group consisting of poly(vinyl pyrrolidone) and poly(ethylene glycol).

26. The method of claim 1-24 or 25 wherein the cyclodextrin is a compound of the Formula I:



Formula 1

wherein:

n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of $R_1 - R_9$ is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, a -O-$(CH_2)_mSO_3^-$ group wherein m is 2 to 6, -$OCH_2CH_2CH_2SO_3^-$, or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

27. The method of claim 26, wherein the formulation further comprises at least one of antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavor or sweetener.

28. A method of unmasking a preservative property of an active agent not otherwise known to possess such property, the method comprising the step of exposing the active

agent to sulfoalkyl ether cyclodextrin in an aqueous solution, optionally contaminated with a microbe, wherein the cyclodextrin is present in an amount sufficient to unmask a preservative property of the drug, and the drug is present in an amount sufficient to provide a preservative effect against a microbe in the solution.

29. The method of claim 28, wherein the preservative property is selected from the group consisting of biocidal activity, growth retarding activity, biostatic activity, and a combination thereof.

30. The method of claim 29, wherein the active agent is a phenol-based active agent.

31. A method of preserving a liquid formulation comprising the step of:

including a sulfoalkyl ether cyclodextrin an optional active agent and a conventional preservative in the formulation wherein neither the cyclodextrin nor preservative is present in a soluble amount sufficient to preserve the formulation independently, but wherein the cyclodextrin and preservative cooperate to provide a preservative effect in the formulation.

32. The method of claim 31, wherein the formulation is preserved by at least reducing the rate of increase of microbial bioburden that may be present in the formulation during storage for at least a predetermined period of time.

33. The method of claim 32, wherein the cyclodextrin is present in an amount less than about 30% wt/vol based upon the final volume of the formulation.

34. The method of claim 32, wherein the preservative and cyclodextrin together provide growth retarding, biostatic and/or biocidal properties to the formulation.

35. The method of claim 32, wherein the preservative and cyclodextrin together at least reduce the rate of bioburden increase in the formulation as compared to a control formulation excluding the cyclodextrin and/or preservative.

36. The method of claim 32, wherein the preservative and cyclodextrin together at least maintain a substantially constant bioburden in the formulation during storage of the formulation.

37. The method of claim 32, wherein the preservative and cyclodextrin together at least reduce the bioburden of the formulation during storage of the formulation.

38. The method of claim 32, wherein the active agent is present and the cyclodextrin is present in molar excess over the active agent.

39. The method of claim 38, wherein a major portion of the active agent is not complexed with the cyclodextrin.

40. The method of claim 38, wherein a major portion of the active agent is complexed with the cyclodextrin by formation of an inclusion complex therewith and/or formation of a salt therewith.

41. The method of claim 38, wherein the cyclodextrin increases the concentration of dissolved active agent in the formulation.

42. The method of claim 32, wherein the formulation has a water activity of less than about 0.97±0.025.

43. The method of claim 32, wherein the formulation has a water activity of less than about 0.95±0.025.

44. The method of claim 32, wherein the formulation has a water activity of less than about 0.90±0.025.

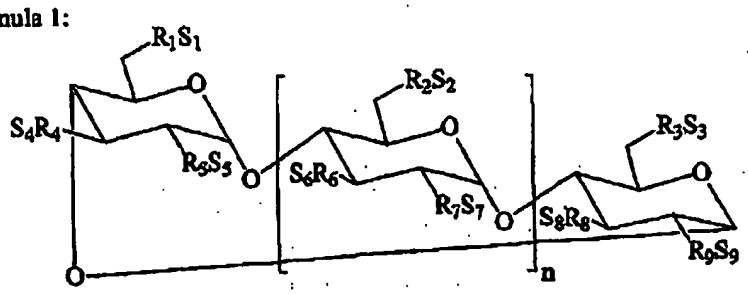
45. The method of claim 32, wherein the formulation has a water activity of less than about 0.85±0.025.

46. The method of claim 32, wherein the formulation has a water activity of less than about 0.80±0.025.

47. The method of claim 32, wherein the preservative and cyclodextrin together preserve the formulation at least against proliferation of one or more of *P. aeruginosa, E. coli, S. aureus, C. albicans*, and *A. niger*.

48. The method of claim 32 wherein the formulation comprises one or more water activity reducing agents, and the one or more water activity-reducing agents is selected from the group consisting of poly(vinyl pyrrolidone) and poly(ethylene glycol).

49. The method of claim 31-47 or 48, wherein the cyclodextrin is a compound of the Formula 1:



Formula 1

wherein:

n is 4, 5 or 6;

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 are each, independently, -O- or a-O-(C_2 - C_6 alkylene)-SO_3^- group, wherein at least one of R_1 – R_9 is independently a -O-(C_2 - C_6 alkylene)-SO_3^- group, a -O-$(CH_2)_mSO_3^-$ group wherein m is 2 to 6, -$OCH_2CH_2CH_2SO_3^-$, or-$OCH_2CH_2CH_2CH_2SO_3^-$); and

S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8 and S_9 are each, independently, a pharmaceutically acceptable cation.

50. The method of claim 49, wherein the formulation further comprises at least one of antioxidant, buffering agent, acidifying agent, alkalizing agent, colorant, solubility-enhancing agent, complexation-enhancing agent, electrolyte, glucose, stabilizer, tonicity modifier, bulking agent, antifoaming agent, oil, emulsifying agent, cryoprotectant, plasticizer, flavor or sweetener.

51. A method of preserving a liquid formulation comprising the step of:

including a cyclodextrin and/or cyclodextrin derivative, an optional active agent, and a water activity reducing agent in the formulation, wherein neither the cyclodextrin nor the water activity reducing agent is present in a soluble amount sufficient to preserve the formulation independently, but wherein the cyclodextrin and water activity reducing agent cooperate to provide a preservative effect in the formulation.

52. The method of claim 51, wherein the cyclodextrin derivative is sulfoalkyl ether cyclodextrin.

53. The method of claim 51, wherein the cyclodextrin is alpha, beta or gamma cyclodextrin.

STATEMENT UNDER ARTICLE 19 (1)

Applicants have amended claims 1, 22, 31 and 51. Support for the added subject matter is found in the application as filed (page 24, lines 5-6; page 37, lines 4-10).

Applicants submit that none of the cited references discloses or suggests that a sulfoalkyl ether cyclodextrin (SAE-CD) possesses a preservative property or that SAE-CD can be a preservative in a pharmaceutical formulation.

Kim et al. disclose a ziprasidone-containing formulation comprising 40% wt. SAE-CD. They do not disclose that such formulation is preserved against microorganisms. They require the use of sterile aqueous solutions in preparing their formulation, so there is no indication of the presence of microbial bioburden in their formulations or of the ability of their formulation to sustain microbial growth. The claims now require a liquid formulation that is "capable of sustaining microbial growth".

Schmidt et al. disclose formulations containing a cyclodextrin complexed with a water insoluble biocide. Their disclosure is not relevant to claims 51, 53-70. Claims 31-50, however, concern the combination of SAE-CD and a conventional preservative in a liquid formulation. They (col. 2, lines 40-65) require that the biocide be present in a soluble amount sufficient to preserve the solution. So, the biocide is present solubilized in solution in an

amount sufficient to, on its own, preserve the solution. The cyclodextrin is present as a solubilizing agent for the biocide so that the biocide can express its biocidal properties. They do not disclose a formulation wherein the cyclodextrin synergistically enhances the biocidal activity of the biocide or vice versa, and they do not suggest that the cyclodextrin has any preserving activity on its own. Claims 31-50 require that the cyclodextrin and biocide are each present in amounts insufficient to individually render preservative activity, and it is the combination that possesses preserving activity.

Stella et al. state that their SAE-CD derivatives have low toxicity (Col. 3, lines 9-14). Therefore, they disclose that SAE-CD is unable to disrupt cellular membranes, meaning it has no cellular toxicity, and microbes are cellular organisms. They also disclose the general use of conventional preservatives in virtually all of the formulations suggested. They had no recognition that SAE-CD can be used in the preparation of a self-preserved formulation. Their formulations were not self-preserved in the absence of conventional preservative. While their formulations include SAE-CD, they do not include "SAE-CD present in an amount sufficient to preserve the formulation."

Applicants note that claims 9, 48, and 51-72 further require including one or more water activity-reducing agents in the formulation. In claims 51-72, the water activity-reducing agent and the SAE-CD synergistically, not individually, provide preservative activity. None of the cited references disclose the combined use of a water activity-reducing agent and SAE-CD in order to provide a self-preserved liquid formulation.

Accordingly, the claimed combination is novel over and provides an inventive step over the art of record.

The undersigned hereby requests that this Statement be fully considered and entered into the above-captioned International Application.

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